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Review

MOLECULAR BREEDING FOR ABIOTIC STRESS TOLERANCE: DROUGHT PERSPECTIVE

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Summary: Drought is the major cause of historic and modern day agricultural productivity losses throughout the world. Drought stress is a complex phenomenon and so is drought tolerance. In addition to genetic conditioning of the traits, environmental effects are difficult to account for precisely. Attempts to generate plant varieties with improved drought tolerance, using selection based breeding strategies, have proved largely unsuccessful. Therefore, progress in breeding for drought tolerance has consequently been limited. Molecular biology, however, provides some means that promise better understanding of the mechanisms of drought stress and drought tolerance. New techniques for evaluating, dissecting and mapping components of drought tolerance as well as the transfer of this information among species are accelerating the understanding of this phenomenon. Ultimately, this could lead to marker-assisted breeding for drought tolerance in some crops. Improved drought tolerance is associated with many potential benefits for maintenance of rural livelihoods in developing countries, income generation and enhanced environmental health. As with many other applications of biotechnology to agriculture, the development of drought tolerant crop cultivars is at the research stage.

Keywords: Drought stress, drought escape, drought avoidance, drought tolerance, mapping populations, recombinant lines, marker tests, statistical analysis, QTL analysis, molecular markers

Introduction

World population is 6.5 billion and is expected to be at least 9 billion by 2050. World food production is limited primarily by environmental stresses. It is very difficult to find 'stress free' areas where crops may approach their potential yields. Abiotic environmental factors are considered to be the main source (71%) of yield reductions [1]. The estimation of potential yield losses by individual biotic stresses in different environments is 14% (insect pests), 28% (diseases and weeds), and 58% by other factors, while abiotic stresses are estimated at 17% (drought), 20% (salinity), 40% (high temperature), 15% (low temperature) and 8% by other factors [2]. It has been estimated that 90% of arable land experience, different environmental stresses, singly or in combination [4]. Drought is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression [5]. It continues to be a challenge to agricultural scientists in general and to plant breeders in particular, despite many decades of research. It is a permanent constraint to agricultural production in many developing countries, and an occasional cause of losses of agricultural production in the developed ones. Conscious selection of desired genotypes by the

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farmers at an early stage, together with natural selection, increased the diversity and created the rich gene pool; a source of variation found today in crop plants [6]. The prediction is that the drought stress, in the form of unpredictable changes in rainfall or competition for freshwater with growing urban populations, will continue to be the major single abiotic factor likely to affect crop yields globally [4]. Wheat production suffers from variability in yield from year to year and from location to location. One of the main environmental abiotic stresses that is responsible for yield instability and limitations in wheat is drought stress, which affects practically every aspect of plant growth and metabolism. Improvement of productivity of crop plants under drought conditions becomes one of the important breeding program objectives. Breeding for drought tolerance is a major objective in arid and semiarid regions of the world due to inadequate precipitation, shortage of irrigation water and high water demand for crop evapotranspiration in such climates. More recently, genetic, molecular and genomic type of studies have revealed other mechanisms that control and regulate plant responses to abiotic stress conditions [7,8]. This review is intended to throw light on the complexity of the drought stress, plant responses and the techniques being used to solve the problem.

How plants Combat Drought

Phenotype is the result of genotype and environmental interaction. Therefore, assessment of desired genotypes is highly dependent on proper environmental conditions. Abiotic stresses (particularly drought, high temperature, salinity and others) generally reduce crop productivity. These stresses are location-specific, exhibiting variation in frequency, intensity and duration. Stresses can occur at any stage of plant growth and development, thus illustrating the dynamic nature of crop plants and their productivity. Drought is the primary abiotic stress causing not only differences between the mean yield and potential yield but also causing variation from year to year, resulting in yield instability. Although selection for genotypes with increased productivity in drought-prone environments has been an important aspect of many plant breeding programs, the biological basis for drought tolerance is still poorly understood. Also, drought stress is highly heterogenous in time, space, degree of stress, growth stage and time of stress exposure [9] and is unpredictable. Due to their secondary mode of life, plants resort to many adaptive strategies in response to different abiotic stresses such as high salt, dehydration, cold and heat, which ultimately affect the plant growth and productivity [10]. Against these stresses, plants adapt themselves by different mechanisms including change in morphological and developmental pattern as well as physiological and biochemical responses [11]. Drought tolerance comprises drought escape (the ability of a plant to escape periods of drought, especially during the most sensitive periods of its development), drought avoidance (the ability of a plant to withstand a dry period by maintaining a favorable internal water balance under drought) and drought tolerance mechanisms (the ability of a plant to recover from a dry period by producing new leaves from buds that were able to survive the dry spell) [12].

Drought escape

Drought escape through early flowering and/or short growth duration is advantageous in environments with terminal drought stress and where physical or chemical barriers inhibit root growth [12,13,14]. On the other hand, later flowering can be beneficial in escaping early-season drought, if drought is followed by rains [15]. Under non-stress conditions, late-flowering varieties tend to yield higher than the early-flowering ones [13,15]. This is because the early-flowering varieties are likely to leave the yield potential unutilized [16].

Drought (or dehydration) avoidance

Dehydration avoidance can be defined as the plant's ability to retain a relatively higher level of 'hydration' under conditions of soil or atmospheric water stress [12]. Levitt [17] recognized two plant types in respect to dehydration avoidance: plants that avoid dehydration by reduced transpiration ('water savers') and plants that use means other than reduced transpiration ('water spenders'). Important features of these are root characteristics (increased water uptake), leaf and stomata characteristics (reduced water loss) and osmotic adjustment to lower the osmotic potential [12,18].

Drought tolerance

Dehydration tolerance describes the ability of plants to continue metabolizing at low leaf water potential and to maintain growth despite dehydration of the tissue or to recover after release from stress conditions. According to Hsiao [19] and Boyer [20], translocation is one of the more dehydration-tolerant processes in plants. It would proceed at levels of water deficit sufficient to inhibit photosynthesis. Ample information has been accumulated in the cereals to show that grain growth is partially supported by translocated plant reserves stored mainly in the stem during the pre-anthesis growth stages. When water stress occurs and the current photosynthetic source is inhibited, the role of stem reserves as a source for grain filling increases, both in relative and absolute terms. Stem reserves may therefore be considered as a powerful resource for grain filling in stress-affected plants during the grain filling stage.

Conventional plant breeding for drought tolerance

Drought is one of the major limitations to food production worldwide and is endemic particularly in the semiarid tropics. Improving drought tolerance and productivity is one of the most difficult tasks for cereal breeders. The difficulty arises from the diverse strategies adopted by plants themselves to combat drought stress depending on the timing, severity of stress and stage of crop growth. The problem becomes more complicated by the fact that many loci show efficacy only in a subset of circumstances [21,22,23,24,25]. Breeding for drought tolerance is only one of several options to address the problem. Selection for drought tolerance, while maintaining maximum productivity under optimal conditions, has been difficult [26]. Management techniques are important for improving water capture and conservation. Crops can be sown during particular periods so that critical development stages do not occur when there is low moisture availability. It has been reported that photosynthesis and several other related physiological traits differ significantly between drought-tolerant and susceptible genotypes. Some crops are naturally more drought tolerant than others, and are obviously better suited to drought environments. Drought tolerance is a complex trait, and breeding for tolerance has been hampered by interactions between genotype and environment resulting from variation and intensity of rainfall from year to year. From the conventional plant breeding point of view, several characteristics and prcocesses have been considered important in drought tolerance improvement (Fig. 1). Similarly, many physiological and morphological (phenotypic) characters are considered important in adaptation to drought stress. Osmotic adjustment, in which the plant increases the concentration of organic molecules in the cell water solution to bind water is one example of a mechanism that alleviates some of the detrimental effects of drought. A thicker layer of waxy material at the plant surface and more extensive and deeper rooting are the others. Root development plays a major role in a plant's response to water availability. Root development is restricted in acid soils, because of aluminium toxicity. Phosphorus is also highly

fixed in acid soils and this too adversely affects root development. Therefore, improving aluminium tolerance and phosphorus uptake indirectly improves drought tolerance. Similarly, physiological and biochemical traits that might enhance drought tolerance have been proposed but only a few of these mechanisms have been demonstrated to be casually related to the expression of tolerance under field conditions [15].

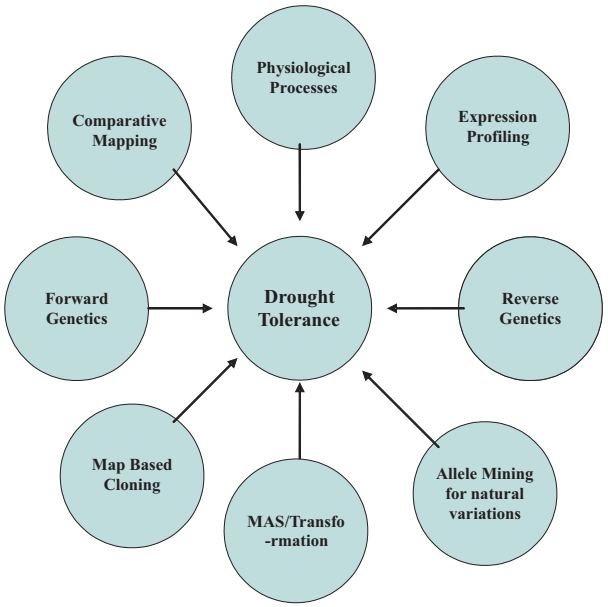

Fig. 1. Drought tolerance improvement; tools and processes. Multidisciplinary efforts require combination of physiological, genetic and genomic approaches to combat abiotic stresses such as drought stress.

Molecular plant breeding for drought tolerance

Conventional breeding methods can be used to enhance levels of drought resistance for many crop species. However, due to the complex nature of the trait and the complicating effects of the environment, progress is not as rapid as for simpler traits. Consequently, there is hope that methods of molecular biology might be used to make breeding more efficient and effective. Although molecular breeding promises much, there has been relatively little progress to date in breeding for drought resistance. There is lack of knowledge about the processes between the DNA sequence of a gene, and a trait (phenotypic gap). There are several ways to reduce this information gap. These ways gradually reveal the functions of the genes and their connection with the phenotypes. Identification of areas of the genome that have a major influence on drought tolerance, so-termed QTL, could allow marker assisted selection (MAS) to be used to identify those plants from a population that are likely to be better adapted to drought. These areas of the genome are invariably numerous and large, and it is a further step to identify the genes underlying the QTL and assess their contributions to drought tolerance (Fig. 2). In addition to accounting for variation in drought tolerance directly, these QTL will also largely determine root morphology and development, and may well govern expression of a whole range of other associated genes. Once the major QTLs have been identified, they might be transferred among plants using linked molecular markers associated with them.

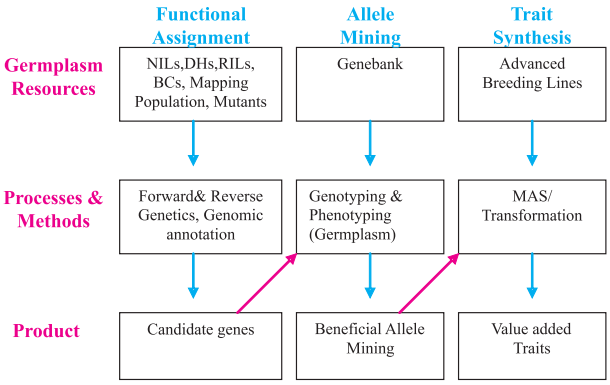

Fig. 2. Trait capture to gene Discovery. With this information, scientists have two choices; either to move the identified chromosome segment into elite breeding lines or clone the gene in the segment that is actually responsible for the desired phenotype and manipulate through genetic engineering.

Mapping populations

Mapping is putting markers (and genes or QTL) in order, indicating the relative distances among them and assigning them to their linkage

groups on the basis of their recombination values from all pairwise combinations. Knowledge about the genetic concepts of segregation and recombination is essential to the understanding of mapping. The construction of a linkage map is a process that follows the segregation of molecular markers in a segregating population and put them in linear order based on pairwise recombination frequencies. Thus, a mapping population with high number of polymorphisms over the total genome is highly desirable. Mapping populations consist of individuals of one species or, under special condition, these are derived from crosses among related species where the parents differ in the traits under study. An ideal mapping population consists of the following, (1) the trait under study must be polymorphic between the parent lines, (2) trait heritability must be essentially high and (3) the identification of genetic factors linked to trait in segregating population must be high if the parent lines used for raising the mapping population are extremely different. These prerequisites apply to both qualitative and quantitative traits. Towards this end, various ways have been used to create mapping populations, which are illustrated in Fig. 3. Populations used for mapping are usually derived from F1 hybrids between two lines (either homozygous or heterozygous), which show allelic differences for selected probes. Genetic maps of plants are constructed based on several different kinds of populations [27], with each population structure having unique strengths and weaknesses. Four types of population are commonly used for map construction and mapping experiment. These are F2 population, back cross population (BC), doubled haploid (DH) population, and recombinant inbred lines (RILs). Most genetic mapping populations in plants have been derived from crosses between largely homozygous parents. Different mapping populations used for QTL analysis for drought tolerance in cereals are shown in Table 1.

F2 population

The simplest form of a mapping population consists of F2 plants. Mendel used F1 and F2 populations to lay down the foundation stone of genetics. F2 populations can be quickly developed and harbor all possible combinations of parental alleles [28]. The degree of polymorphism of parents of F2 population can be assessed at phenotypic level (morphological markers) or genetic level by the use of molecular markers. However, each F2 individual has a different genotype and no replication or experimental design can be employed to effectively control environmental influence. F2 populations can not be easily preserved because these plants are not immortal. To solve this problem, evaluation of F3 progenies derived from individual segregants by selfing can be used but gains in precision are partly sacrificed due to genetic heterogeneity [29,30]. A major disadvantage of F2 population is that the data of marker genotypes cannot be repeatedly used.

Back cross (BC) population

This is a widely used mapping population for the analysis of specific DNA fragments derived from one parent of the cross. It is derived by crossing F1 individuals to one of the two parents (recurrent parent). During this process, unlinked donor fragments are separated by segregation and linked donor fragments are minimized due to recombination with the recurrent parent. BC population has similar advantages and drawbacks as F2 populations. Advanced backcross populations are generated by repeating this process several times to restrict the number and size of donor fragments. Use of Backcross population is an important strategy if a single trait has to be introduced into a cultivar that already contains other desirable traits. Another requirement is that the two parents be crossable and produce fertile progeny. A major disadvantage of BC population is that the data of marker genotypes cannot be repeatedly used.

Table 1. Quantitative Trait Loci (QTLs) for drought stress tolerance in cereal osmotic adjustment.

Trait	Species Population	Source	Mapping	Reference
OA and dehydration Tolerance	Rice	CO39 x Moroberekan	RIL	124
Osmoregulation Under drought	Wheat	Songlen x cobdor 4/3Ag14	RIL	125
OA under drought	Barley	Tadmor x Er/Amp	RIL	126
OA under drought	Rice	CT9993 x IR62266	DH	127
OA under water Deficit	Wheat	-	Bread wheat genotypes	128
		Cellular membrane stability (CMS)		
CMS under Drought	Rice	CT9993 x IR62266	DH	129
		Hormonal responses under Drought		
ABA Conc.	Wheat	CS x Ciano67	DH	130
Leaf size & ABA Accumulation	Rice	IR20 x 63-83	F_2	131
Leaf ABA	Maize	OS420 x IABO78	F_3	132
		Salinity Tolerance		
Na^+, K^+ uptake	Rice IR4630 x IR10167	Nona Bokra x Pokkali/	RIL	133
Na^+, K^+ uptake Dry mass, ratio	Rice	IR4630 x IR15324	RIL	134
Na^+, K^+ absorption	Rice	-	RIL	135
		Heat Tolerance		
Heat tolerance at Grain filling	Wheat	Ventor x Kar192	F_1, F_2, F_3	136
		Cold & Chilling Tolerance		
Cold tolerance	Rice	Norin-PL8 x Silewah	NIL	137
Cold tolerance at Booting stage	Rice	Akihikari x koshihikari	DH	138
Photosynthesis under Chilling stress	Maize	Ac7643 x Ac7729/TZSRW	RIL	139
Chilling tolerance	Rice	M202 x IR50	RIL	140

Table 1 contd.

		Mineral Toxicity Tolerance		
Al tolerance	Wheat	Bh1146 x Anahuac	RIL	141
Al tolerance	Rice	Azucena x IR1552	RIL	142
Al tolerance	Maize	Cat-100-6 x S1587-17	F_2	143
Al tolerance	Barley	Yambla x WB229	F_2	144
Al tolerance	Rye	M39A-1-6 x M77A1	RIL	145
Al tolerance (Relative Root Length)	Rice	IR64 x *O. rufipogon*	RIL	146
		Stay Green Character		
Stay green Chlorophyl Content	Sorghum	B35 x TX430	RIL	147
Stay green under Drought	Sorghum	QL39 x QL41	RIL	148
Stay green Chlorophyl Content	Sorghum	B35 x TX7000	RIL	149
Stay green pre-Flowering drought	Sorghum	SC56 x TX7000	RIL	150
Stay green	Sorghum	B35 x TX7000	RIL	151
Stay green	Sorghum	IS9830 x E36-1 N13 x E36-1		
Stay green; Chlorophyl Content	Rice	Mutagenesis (Hwacheong-wr)	Mutant	152
		Root/Shoot Responses Under Drought		
No. of Tiller & root, Dry weight, thickness	Rice	CO39 x Moroberekeп	RIL	153
No. of Tiller & root, Penetration ability	Rice	CO39 x Moroberekeп	RIL	154
Root morphology & Distribution	Rice	IR64 x Aucena	DH	155
Root length, number Thickness, penetration index	Rice	IR58821 x IR52561	RIL	156
Root penetration Ability	Rice	Bala x Azucena	RIL	157
Root thickness, root Penetration index	Rice	IR64 x Azucena	DH	158
Root thickness, root Penetration index	Rice	CT9993 x IR62266	DH	127
Yield & root traits Under limited water	Rice	IR64 x Azucena	DH	159
Root traits	Rice	CO39 x Moroberekeп	DH	160
Root traits & yield	Maize	Lo964 x Lo1016	F_3	161
Root traits	Rice	IR1552 x azucena	RIL	162

Double haploids (DH) population

DH population can also be called permanent populations for mapping purposes and are ideal crossing partners in raising mapping populations because they are almost free of residual heterogeneity. Producing wheat haploids by crossing bread wheat with maize or pearl millet has become a significant procedure. Double haploids are commonly used in many plant species, which are amenable to anther or microspore culture (from F1 plants). This is followed by chromosome doubling by colchicine treatment,which prevents the formation of spindle apparatus during mitosis, thus inhibiting the separation of chromosomes and leading to double haploid cells. Because the plant has two identical homologues, the amount of recombinational information is exactly equivalent to a backcross. However, DH individuals are completely homozygous and can be self-pollinated to produce large numbers of progeny, which are all genetically identical. This permits replicated testing of phenotypes and also facilitates distribution of identical DH populations. A major disadvantage of DH population is that, it is not possible to estimate dominance effects and related types of epistasis, and the rates of pollens or microspores successfully turned into DH plants may vary with genotypes, thus causing segregation distortion and false linkage between some marker loci.

Recombinant inbred lines (RILs) or single seed descent (SSD) population

Homozygous or 'permanent' populations can also be made by traditional means i.e., by selfing or sib-mating individuals for many generations starting from F2 by the single seed descent (SSD) approach until almost all of the segregating loci become homozygous. Each of the loci having allelic difference in parents has two genotypes with equal frequencies. However, genetic distances based on RIL population are enlarged compared to those obtained from F2, BC or DH populations, because many generations of selfing or sib-mating will increase the chance of recombination. Consequently, RIL populations show a higher resolation than maps generated from F2 populations. In plants, self pollination allows the production of RILs in a relatively short number of generations. In fact, within six generations, almost complete homozygosity can be reached. Also, the RIL populations have several advantages, including reproduction, which favor the genetic analysis of quantitative traits because experiments can be replicated over years and locations; and the use of dominant marker types with the same efficiency as the co-dominant ones [31]. A major shortcoming of RIL populations is that development of RIL population takes long time and it is not possible for all individuals to be homozygous at all segregating loci through limited generations of selfing or sib-mating, which decreases the efficiency for QTL mapping to some extent. Also, replicated testing is possible as with DHs.

Mapping quantitative trait loci (QTLs)

Characters exhibiting continuous variation are termed quantitative traits. Quantitative traits show continuous phenotypic variation in a population resulting from the combined allelic effects of many genes and environmental conditions [32]. In crop plants, most traits of agricultural and economical significance exhibit quantitative inheritance, such as yield, plant maturity, disease resistance and stress tolerance. The genetic loci, which control quantitative traits, are referred to as QTL (quantitative trait loci). QTL analysis has been a major area of genetical study for many decades. The earliest documented experiments on linkage analysis between quantitative effects and marker genotypes have been reported by Sax [33] and Thoday [34]. However, for most of the period up to 1980, the study of quantitative traits has largely involved biometrical approaches

based on means, variances, covariance of relatives. Consequently, very little was known about the biological nature of quantitative or natural variation in terms of number and location of the genes that underlie them [35,36,37]. It is only during the past decade, with the appearence of efficient molecular marker technologies and specific statistical methods, that it became possible to follow the segregation of quantitative traits via linked markers [39] and to detect effects, numbers and map positions of QTL.

Statistical methods for mapping and analysis of quantitative trait loci (QTL)

Traditional genetic studies have concentrated on dichotomous traits such as the presence or absence of a disease resistance in plants. Such traits are often the result of a mutation at a single gene. However, most of the agronomically important traits exhibit a continuous range of phenotypic variation, which is more or less normally distributed [39] and can be explained by the independent action and potential interaction of many discrete genes affected by environmental factors [40]. The precise number of genes involved is usually not known [37]. A major gene affecting a quantitative trait that has been localized to a chromosome is called a quantitative trait locus (QTL) [41]. Before 1980, classical quantitative genetics was mainly based on statistical techniques, such as means, variances and covariances of relatives, with no knowledge of the number and location of the genes that underlie them [42]. The first report of an association between a morphological marker locus and a quantitative trait was reported by Sax [33] (between a pigment locus and seed size in the bean, *Phaselous vulgaris)*, demonstrating that the variation of size differences of the seed-coat followed the fundamental Mendelian properties of segregation and recombination. A key development in the field of complex trait analysis was the establishment of large collections of molecular and genetic markers, which offered the possibility of mapping QTLs depending on the level of resolution and density of the genetic maps. Recent and continuing advances in molecular genetics and statistical techniques make it possible to identify the chromosomal regions where these QTLs are located [38].

The statistical analyses of associations between phenotype and genotype in a population to detect quantitative trait loci include single-marker mapping [43,44], interval mapping [45], and composite interval mapping (CIM) [46,47], plus multiple trait mapping [48,49].

Single marker tests

It is useful to start analysis of the genetics of a quantitative trait by testing for associations between the trait values and marker genotypes. The simplest method for QTL mapping is single-marker mapping, including *t*-test, and analysis of variance (ANOVA) and simple linear regression, which assess the segregation of a phenotype with respect to a marker genotype [50]. Accordingly these principles classify progeny by marker genotype, and compare phenotypic mean between classes (*t*-test or ANOVA). A significant difference indicates that a marker is linked to a QTL. The difference between the phenotypic means provides an estimate of the QTL effect. This approach can indicate which markers linked to potential QTLs are significantly associated with the quantitative trait investigated. In short, QTL location is indicated only by looking at which markers give the greatest differences between genotypic group averages. Depending on the density of markers, the apparent QTL effect at a given marker may be smaller than the true QTL effect as a result of recombination between the marker and the QTL. The advantage of this method is the simplicity of procedure that can be accomplished by a standard statistical analysis software package, such as SAS and Minitab. In contrast, the main weakness of the single-marker tests is the failure to provide an

accurate estimate of QTL location or recombination frequency between the marker and the QTL, because the evaluation of individual markers is done independently, and without reference to their position or order [51].

Single interval mapping (SIM)

Interval mapping is probably the most familiar method of QTL analysis. The introduction of interval mapping offered a new strategy to discern weak effects from genetic distance between marker locus and putative QTL using the power of a complete genetic map. The intervals that are defined by ordered pairs of markers are searched in increments, and statistical methods are used to test whether a QTL is likely to be present at the location within the intervals or not. The principle behind interval mapping is to test a model for the presence of a QTL at many positions between two mapped marker loci. The model fit, and its goodness is tested using the method of maximum likelihood. If it is assumed that a QTL is located between two markers, the 2-locus marker genotypes contain mixtures of QTL genotypes each. Maximum likelihood involves searching for QTL parameters that give the best approximation for quantitative trait distributions that are observed for each marker class. Models are evaluated by computing the likelihood of the observed distributions with and without fitting a QTL effect. The LOD (logarithm of the odds) score is the log of the ratio between the null hypothesis (no QTL) and the alternative hypothesis (QTL at the testing position). Large LOD scores correspond to greater evidence for the presence of a QTL. The best estimate of the location of the QTLs is given by the chromosomal location that corresponds to the highest significant likelihood ratio. The LOD score is calculated at each position of the genome. In the case of many missing genotypes and large gaps on the map, the missing data are replaced by probabilities estimated from the nearest flanking markers [52]. Until now, many software packages based on interval mapping were developed for QTL mapping, such as MAPMAKER/QTL [53] and QGene [54]. In comparison to single marker mapping, the benefits of these programs are a curve available across the genetic map, indicating the evidence of QTL location, and which allows the inference of QTLs to positions or gaps between two markers in order to make proper analysis for incomplete marker genotype data. Meanwhile, analysis can be used for testing the presence of genotyping errors [55].

Composite interval mapping (CIM)

There are two problems with single interval mapping (SIM) method resulting from the single QTL model mentioned above. One is that the effects of additional QTL will contribute to sampling variance. The other is that the combined effects of two linked QTLs will cause biased estimates. The ideal solution would be to fit a model that contains the effects of all QTL. However, the tremendous number of potential QTLs and their interactions will lead to innumerable statistical models and heavy computational demands for using statistical approaches to locate multiple QTL. To deal with this problem, several key papers have been published [46,47,56,57].The approach of composite interval mapping assesses the probability that an interval between two markers is associated with a QTL that affects the trait of interest, as well as controlls for the effects of other background markers on the trait. In theory, CIM gives more power and precision than SIM because the effects of other QTLs are not present as residual variance. Furthermore, CIM can remove the bias that would normally be caused by QTLs that are linked to the position being tested. The key problem with CIM concerns the choice of suitable background markers to serve as covariates.

Approaches used for QTL mapping

The identification of QTL for economically important traits has been achieved primarily by two approaches, either through linkage mapping to anonymous markers or through association studies involving candidate genes.

QTL analysis through a molecular marker approach

The principle of QTL mapping is to associate the phenotypically evaluated trait(s) with molecular markers using statistical tools. The map locations of QTL can then be estimated by the means of highly associated markers. Typically, the detection and location of the loci underlying quantitative trait variation involves three essential steps. First, a segregation population is created and characterized with molecular markers. This usually leads to the construction of a genome wide genetic map of the population. Second, the individuals of the same population are phenotypically evaluated for the traits under investigation. Finally, genotypic molecular markers are analyzed for association with the phenotypic trait data using appropriate statistical methods. This type of QTL analysis can lead to the elucidation of QTL parameters in terms of number, position, effects and interactions between them. Association of morphological markers with quantitative traits in plants was observed quite early [33,58], and the first steps towards mapping of QTLs or polygenes were taken based on the scarce markers available [34]. Currently, complete genetic maps exist for many crop species and algorithms have been developed for QTL mapping in a wide range of pedigrees [59]. The simplest methods were based on single marker analysis, where the differences between the phenotypic means of the marker classes compared using F-statistics, linear regression or nonparametric tests [33,43,60]. The computer program Mapmaker [28] has been used extensively for performing interval mapping in plant studies. Interval mapping, now called simple interval mapping (SIM), searches for a single target QTL throughout a mapped genome.

QTL analysis through a candidate gene approach

The candidate-gene approach is a powerful and robust method. Compared to the genome wide mapping strategy, the chances of finding markers linked to putative QTL are maximized, since the selection of candidate-gene markers is based on known relationships between bio-chemistry, physiology and the agronomic character under study. This approach has been applied successfully in various QTL analyses, such as mapping QTL for defense response to diseases in wheat [61,62], for resistance to corn earworm in maize [63,64] and early growth traits in maize [65].

Conclusions from QTL mapping experiments for abiotic stress

In the traditional models of quantitative genetics simplifying assumptions were made about equality and strict additivity of gene effects [32]. From the results of the QTL mapping experiments, it has become clear that such assumptions are incorrect. In many mapping experiments, a relatively small number of QTLs accounts for very large portions of phenotypic variance, with increasing numbers of genes accounting for progressively smaller portions of variance, until the significance threshold is reached [59]. The number of QTLs located for particular traits in individual studies varies from one to sixteen, usually being below five [39]. The proportion of phenotypic variation explained by each QTL and all QTLs together depend on heritability of the trait as well as on the portion of revealed QTLs. QTLs are usually spread over all chromosomes, but clusters of QTLs in certain chromosomal regions have been observed as well. Differences occur in QTL incidence when quantitative traits are scored in many environments

or during many years. However, comparative studies between related species have revealed conservation not only in marker order but also in locations of some QTLs [66]. Examples of QTL studies for different traits related to drought tolerance in various mapping crosses of cereals are shown in Table 1.

Applications of molecular markers

The invention of molecular marker technology such as RFLP, RAPD, AFLP, and SSR has opened up a new era for genetic analysis of plant genomes. Genetic mapping using molecular marker technology is of great significance to plant breeding, plant genetics and evolutionary studies. The most common applications of genetic linkage maps are concentrated on the following areas. First, genetic linkage maps can be used for marker-assisted selection (MAS) in plant breeding. They could help to identify DNA markers linked to single genes of major agronomic importance and the tightly linked DNA markers can be used as diagnostic tools for MAS (Table 2). This is particularly suitable and powerful for screening for monogenic disease resistance. One of the successful examples is MAS for soybean cyst nematode resistance (*SCN*) [67]. The SSR marker *Satt309*, which is located 1-2 cM away from the gene *rhg1* for resistance to *SCN*, has been developed and used for tagging and tracking the gene through breeding programs, leading to the development of resistant lines. The use of SSR markers has largely decreased the time and effort involved as compared to phenotypic selection. Second, genetic linkage maps can be used for the genetic analysis of quantitative traits. With the construction of molecular linkage maps, characterization of quantitative traits has been greatly facilitated in identifying the genomic regions responsible for the traits and estimating the possible number of genetic factors controlling the traits of interest [38]. Third, genetic linkage mapping can be used to correlate the phenotypic traits with the genes controlling the trait, which includes map-based cloning of a gene of known heritable phenotype and postulating candidate genes for a trait with known biochemical basis. Finally, genetic linkage maps provide insights into chromosomal organization and could be useful in map-based evolutionary studies by comparative mapping.

Linkage maps

Construction of a genetic linkage map is based on observed recombination between marker loci in the experimental cross. Segregating families, e.g., F2 population or BC progenies, DH population or RIL lines are commonly used. In barley, the use of double, haploid progenies produced from the F1 generation simplifies genetic analysis. Double haploid lines have undergone only one meiotic cycle and carry a completely homozygous chromosome set. This means that the genetic information per plant is constant, irrespective of the marker system used [68]. Genetic map distances are based on recombination fractions between loci. The Haldane [69] or Kosambi [70] mapping functions are commonly used for converting the recombination fractions to map units or centiMorgans (cM). The Haldane mapping function takes into account the occurrence of multiple crossovers but the Kosambi mapping function accounts also for interference, which is the phenomenon of one crossing-over inhibiting the formation of another in its neighborhood [71]. Computer programs performing full multipoint linkage analysis include Mapmaker [28] and JoinMap [72]. Linkage map of human genome based on segregation analysis of 814 (CA)n microsatellite loci was initially constructed [73].

However in plants, mapping with STMS markers did not reach this level of resolution so far [74], although the very first attempt to map sequence-tagged microsatellite sites (STMS) loci in any species was made as early as 1992, in rice using (GGC)n microsatellites [75,76]. Several

barley maps based on SSRs [77] and randomly amplified SSRs [78] have been developed. Mapping of the whole genome using microsatellite loci is also currently in progress in many crops i.e. *Brassica* [79], soybean [80] and maize [81]. Microsatellite loci, other than STMS markers, have also been used for mapping in different plant species. In bread wheat, two microsatellite maps, one with 279 loci [82] and another with 50 loci [83] have been prepared. Also in tetraploid wheat, 14 microsatellite loci were mapped on chromosomes 5A and 5B, which carry genes for protein content, vernalization response and resistance to Hessian fly. Utilizing International Triticeae Mapping Initiative (ITMI) population, an integrated map of wheat genome (with 1200 RFLP earlier mapped) [84,85] became available, to which 279 *gwm* microsatellite loci were added [82]. Later, Gene and Genome Mapping Group, Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany, has successfully assigned a set of another 70 microsatellite loci to specific chromosomes using nulli-tetrasomic lines [86]. Additional microsatellite loci have been mapped by Leroy [87]. Comparative mapping within the *Poaceae* family has also revealed high levels of conservation of gene order [88].

Mapping qualitative traits for marker assisted selection (MAS)

Qualitative genes are inherited in a Mendelian fashion and their allelic forms give qualitatively distinct phenotypes. The phenotypes in a segregating progeny can be scored in a similar fashion as molecular markers. A normal segregation analysis will reveal linkages to any of the markers. Mapping a gene to a certain location on the chromosomes demands a linkage map of the whole genome, but genes can also be tagged with molecular markers without any previous information of the map location of markers used. Two approaches have been proposed for this purpose, i.e., use of near-isogenic lines, NILs [89,90], and pooled DNA samples [91]. NILs differ only by the presence or absence of the target gene and a small region of flanking DNA.

Hundreds of arbitrarily primed PCR-based markers can easily be screened to identify differences between isogenic lines, and these differences are likely to be linked to the target gene. The NILs have been used in barley to tag a powdery mildew resistance gene [92] and a spot blotch resistance gene [93]. In bulked segregant analysis (BSA), DNA pools of individuals of a crossing progeny are made based on their phenotype and screened for differences in the molecular markers [91]. BSA has successfully been used in barley for tagging several disease resistance genes with RAPD markers locating 1.6-12 cM from the target locus [94,95,96,97]. Also, BSA has been proposed for tagging quantitative loci with a major effect: theoretically QTL alleles with phenotypic effects of 0.75-1.0 standard deviations should be detectable in DH populations of 100-200 lines [98].

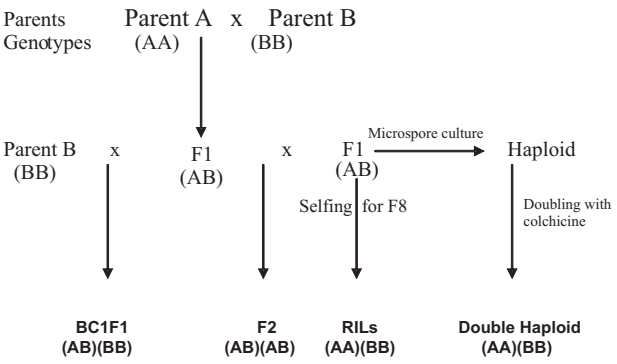

Fig. 2. General strategies for the construction of mapping populations for trait capture.

The first example of a gene linked to a microsatellite (AT) was a soybean mosaic virus resistance gene (*Rsv*) [99,100]. Several other resistance genes including those for resistance to peanut mottle virus (*Rpv*), *Phytophthora* (*Rps3*) and Javanese root knot nematode, were found to be clustered in the same region of

Table 2. Tagging of QTLs of different abiotic stresses in wheat using molecular markers.

Stress	Marker	Reference
Boron Tolerance	RFLP	163
Sprouting Resistance	RFLP	164
Cold Tolerance	RFLP	165
Preharvest Sprouting Tolerance	STS, SSR	120
	RFLP	166
Vernalization	RFLP	167
	RFLP	168
	RFLP, STMS	103
	RFLP	169
	STMS	170
Aluminium Tolerance	RFLP	171
ABA Production & Response	RFLP	130
Salt Tolerance	Protein markers	172
NA^+/K^+ Discrimination	RFLP	173
Frost Tolerance	SSR, RFLP	106
Drought Stress	RFLP, AFLP, SSR Morphological and Biochemical markers	174

soybean genome where this (AT)n microsatellite was found to be associated with *Rsv.* Microsatellite markers, associated with soybean cyst nematode (*SCN*) resistance locus, sclerotinia stem rot resistance and brown stem rot resistance, were also reported by [101,102]. In wheat, microsatellite markers have been applied widely for tagging genes or QTLs determining dwarfing [103,104,105], vernalization response [103,106], disease resistance [107,108,109,110,111, 112,113,114,115,116], flour colour and milling yield [117], grain protein content [118,119], preharvest sprouting tolerance [120], grain yield and its components [121,122] and frost [106]. In durum wheat, some microsatellites have been mapped in two regions of chromosome 5A each carrying a QTL, for high grain protein content and for heading [123].

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