CHAPTER 9

CLONING QTLS IN PLANTS

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Abstract: The utilization of a number of genomics platforms and analytical methods allows us to fine map and clone major quantitative trait loci (QTLs) responsible for the genetic control of quantitatively inherited traits. To date, most plant QTLs that were successfully cloned have been dissected by means of a positional cloning approach within a biparental cross. In some cases, an association between allelic variation at a candidate gene and a phenotype has been established through the analysis of existing genetic accessions. The effectiveness of these strategies can be enhanced by using appropriate genetic materials (e.g. introgression libraries, panels of unrelated accessions, etc.) and the latest developments in forward- and reverse-genetic platforms. Under this respect, the ‘omics’ platforms provide a new paradigm to identify candidate genes and clues for their function. Completion of genome sequences and improved bioinformatics will facilitate in silico cross-matching of candidate sequences with QTLs in programmes of positional cloning or association mapping. Several QTLs have been associated to candidate genes solely based on map information and further circumstantial observation, and without completing a formal cloning procedure. Although QTL mapping and cloning have so far been almost synonymous with the dissection of the genetic control of naturally available phenotypic differences, genes involved in controlling quantitative traits could be identified also by combining quantitative genetics with insertional mutagenesis. Although QTL analysis and cloning addressing naturally occurring genetic variation will continue to shed light on mechanisms of plant adaptation, a greater emphasis on approaches relying on mutagenesis and candidate gene validation is likely to accelerate the discovery of the genes underlying QTLs.

1. INTRODUCTION

For most phenotypic traits, variation among individuals within one species cannot be accounted for by allelic differences at one single locus. Instead, the action of multiple loci, their interactions and random environmental effects are involved

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in determining phenotypes. Early work indicated that loci with major effects on quantitative traits could be identified and mapped on chromosomes by evaluating the correlation between trait values and the allelic state at genetic markers (Sax 1923; Thoday 1961). This led to the definition of quantitative trait locus (QTL; Geldermann 1975) as a genetic locus where functionally different alleles segregate and cause significant effects on a quantitative trait. With the advent of molecular marker technology, QTL mapping on chromosome linkage maps has become a standard procedure in quantitative genetics (Paterson et al. 1988; Tanksley 1993; Lynch and Walsh 1998; Hackett 2002). By coupling marker technology with genomics resources such as bacterial artificial chromosome (BAC) libraries and physical maps, and by exploiting appropriately developed plant materials, it is now possible to clone single QTLs and identify the DNA polymorphisms responsible for a target QTL (Paran and Zamir 2003; Salvi and Tuberosa 2005). The impact of QTL mapping and cloning on our understanding of plant biology is remarkable: for the first time, we have the opportunity to unravel and describe the genetic complexity (i.e. the number and the type of action of genes) behind quantitatively inherited processes/traits such as adaptation to photoperiod conditions, extreme environments, domestication and many others, including yield and its stability. Such description is at the core of evolutionary genetics and plant breeding.

This chapter highlights the major methodological trends toward QTL cloning and some preliminary indications on the molecular nature of quantitative variation. Clearly, genetic adaptation also involves selection for mutations with a strong effect on the phenotype which are usually classified as Mendelian genes rather than QTLs. Examples of such loci are the major genes \( \text{FLC} \) and \( \text{Frigida} \) involved in the vernalization response and flowering time of \( \text{Arabidopsis} \) (Michaels and Amasino 1999; Johanson et al. 2000), the vernalization \( \text{Vrn1-3} \) loci in wheat and barley (Yan et al. 2003, 2004, 2006) and the photoperiod response \( \text{Ppd-H1} \) locus in barley (Turner et al. 2005). Because the cloning and the characterization of such loci did not require the QTL mapping and cloning toolbox, the relevant results have not been considered for this review.

2. AVENUES TOWARD QTL CLONING

QTL analysis for a given trait in plants usually begins with a primary (or coarse) QTL mapping step which localizes all major loci responsible for the trait variation observed in a given biparental population. Subsequently, a QTL is mapped within a chromosome supporting interval of ca. 10–30 cM (Lynch and Walsh 1998; Doerge 2002) which can include several hundred genes. The challenge is then to enhance the genetic resolution so that the QTL is confined to a chromosome segment ideally including only one gene. Positional cloning and association mapping are the two main approaches that have been deployed for cloning QTLs. Both approaches exploit linkage disequilibrium (LD; i.e. the level of non-random assortment of alleles at different loci) in order to verify the correlation between the shortest chromosome region tagged by molecular markers and the trait value.