Molecular markers and QTL analysis in crop plants

P. K. Gupta

Majority of quantitative traits in crop plants are controlled by polygenes; most of them have minor effects and only occasionally some of the genes have major effects\(^1\). These gene loci are described as quantitative trait loci (QTL) and can be detected with the help of molecular markers, which should also segregate in a Mendelian manner. A QTL actually describes a region of the chromosome defined either by linkage to an individual molecular marker or by two flanking markers which may or may not be linked with the QTL\(^2\). Molecular markers that are used for QTL analysis largely refer to DNA-based markers, which can be used in plant breeding for various purposes. For instance, marker–trait association has been successfully used for indirect marker-assisted selection (MAS) in cases like soybean cyst nematode (SCN) resistance and rice bacterial blight resistance\(^3,4\). A variety of molecular marker systems are now available for use in QTL analysis, and several laboratories in India are involved in work on molecular markers for crop improvement\(^5\). Elsewhere, we have discussed in some detail the principles, methodology and relative merits of individual marker systems\(^6-9\).

A variety of methods are available for QTL analysis in crop plants\(^10,11\). The earliest and simplest methods for QTL analysis included single-marker regression or tests for independence in segregation of molecular marker and the trait of interest. A serious limitation of this approach is confounding of the effect of one QTL by many others that influence the trait. Another serious limitation is that a QTL with minor effect and loose linkage cannot be distinguished from a QTL with minor effect and tight linkage. A milestone in QTL analysis, therefore, was the QTL interval mapping\(^12\), which had several advantages over the above traditional methods involving regression analysis or tests of independence. This approach needs a molecular genetic map and utilizes, in a single analysis, infor-
mation from all the markers of a linkage group, thus permitting detection of QTL in each interval (described as a bin) lying between any two flanking markers that individually may show no association with the trait. It has been shown that some of the markers showing no association through single-marker analysis, were helpful in detecting QTLs through interval mapping. Modifications of simple interval mapping (SIM) have also been suggested in the form of composite interval mapping (CIM) and multiple interval mapping (MIM), which take care of some of the limitations of SIM. However, the difficulty in using interval mapping as above sometimes lies in the availability (or construction) of a molecular map that must have been prepared using the same mapping population which is used for interval mapping. The unavailability of such a map sometimes forces researchers in this area to use the simple single-marker regression approach discussed above, despite its shortcomings. Several studies on QTL analysis, utilizing the regression approach or independence tests have been conducted in India under the Wheat Biotechnology Network funded by the Department of Biotechnology, Government of India. The limitations of these approaches and those of QTL interval mapping, and the appropriate precautions that need to be observed while using these approaches, have been widely discussed. Unfortunately, these limitations and precautions have been overlooked in some recent studies and are, therefore, briefly discussed here.

In all the above methods of QTL analysis, a mapping population is needed that is derived from a cross between two diverse parents, differing for the character of interest. This mapping population which may consist of F₂, BC₁, doubled haploid or DH lines, recombinant inbred lines or RILs, etc. is used both for recording the data on the character of interest and also for genotyping individual plants/lines of the mapping population using the polymorphic molecular markers. These two sets of data are then used for conducting QTL analysis, whether it is the simple single-marker regression approach or the more sophisticated interval mapping. However, before using these two sets of data for QTL analysis, care needs to be exercised to ensure whether the data is suitable for conducting QTL analysis. For instance, one needs to examine whether the molecular marker whose association with the trait of interest is being examined exhibit any segregation distortion, since this may lead to biased estimate of marker-trait association. Therefore, normally in a mapping population consisting of RILs, one needs to examine that all polymorphic markers should segregate in a Mendelian 1:1 ratio and those which deviate significantly from this expected segregation are rejected and not used for any further analysis of marker-trait association. Similarly, the phenotypic data on the quantitative trait being examined should be tested for normality of distribution, before they are used for any further QTL analysis and in case normality is not present, the data need to be transformed on a scale that will achieve normality of distribution. Both these conditions need to be fulfilled before embarking upon QTL analysis.

In some recent studies in bread wheat, molecular markers have been used in work involving marker-trait association, disregarding completely the presence of segregation distortion of markers. There may be several other studies where the data are not examined for segregation distortion of molecular markers and for normal distribution of the data on quantitative traits being analysed. QTL analysis is a newer area of research and is being sometimes conducted by those without adequate knowledge of genetics and plant breeding. There is a need, therefore, in our country to involve more and more geneticists and plant breeders in the use of molecular tools for plant breeding, since they understand the genetics of the traits and that of the molecular markers and can take into consideration the precautions like those mentioned above while conducting QTL analysis. Alternatively, plant physiologists and biochemists who are involved in studies using molecular marker technology, should associate themselves with geneticists and plant breeders to ensure that no serious errors of the kind discussed above are committed while conducting QTL analysis. Furthermore, the involvement of plant breeders is also necessary, since they are the actual users of the results of molecular marker technology for plant breeding.

12. Lander, E. S. and Botstein, D., Genetics, 1989, 121, 185–199.

ACKNOWLEDGEMENTS. The work was carried out under an ICAR–NATP research project.

P. K. Gupta is in the Molecular Biology Laboratory, Department of Agricultural Botany, CCS University, Meerut 250 004, India. e-mail: pkgupta@ndf.vsnl.net.in