Review Article

Horticultural Biotechnology Research
www.jhcbt.org
doi: 10.5455/jhcbt.2015-07-05

Molecular markers in vegetable improvement

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Received: 05.07.2015
Revised: 29.07.2015
Accepted: 04.08.2015
Published: 04.08.2015

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ABSTRACT

Molecular markers directly reveal the polymorphism at the level of DNA. These are tags that can be used to identify specific genes and locate them in relation to other genes. Genetic mapping of disease resistance genes will help to improve the efficiency of plant breeding program, and it will lead to better understanding of the molecular basis of resistance. However, it requires reliable pathological tests and polymorphic genetic markers in a well-defined segregating population. Two strategies for developing markers for disease resistance genes are; the establishment of genetic maps with localization of major genes and quantitative trait locus; and targeting particular regions. Marker-assisted selection (MAS) is an effective tool for enhancing selection procedure in a breeding program. Therefore, MAS is using in the crop improvement program and also useful for the efficient selection of many resistance genes for pyramiding into a single genotype.

KEY WORDS: Gene-tagging, marker assisted selection, molecular makers, quantitative trait locus, vegetable improvement

INTRODUCTION

There are three different types of markers viz., morphological, biochemical, and molecular. Out of these, molecular markers directly reveal the polymorphism at the level of DNA. There are mainly two types of molecular markers, i.e., Hybridization based or non-polymerase chain reaction (PCR) based marker for example restriction fragment length polymorphism (RFLP) (Botstein *et al.*, 1980). PCR based markers example random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), sequence characterized amplified regions (SCARs) (Michelmore *et al.*, 1991), and amplified fragment length polymorphism (AFLP) (Vos *et al.*, 1995). Molecular markers are used for development of saturated genetic maps; DNA fingerprinting; phylogenetic and evolutionary studies; heterotic breeding; gene tagging and marker assisted selection (MAS). Now various technique of identification for vegetable varieties by molecular markers in tomato (Noli *et al.*, 1999), potato (Ashkenazi *et al.*, 2001), onion, garlic, and related species (Fischer and Bachmann, 2000) has been developed. Molecular markers are linked to major disease resistance in tomato such as *Meloidogyne incognita* (Williamson *et al.*, 1994) and tomato mosaic virus (Sobir *et al.*, 2000).

With the help of molecular markers one can linked to co-dominant markers to those plants that are heterozygous for a resistance gene, and it can be easily identified; it is also possible to introgression of recessive or dominant disease resistance genes in a minimum number of generations (Tanksley *et al.*, 1989). Development of markers also allows the gene pyramiding in multi-resistant genotypes with durable resistance (multigenic resistance) and the analysis of polygenic resistance (Melchinger, 1990). Use of DNA markers for disease resistance genes is important and starting point for cloning the genes and determining their mode of action in crop plants (Martin *et al.*, 1993). In this review, I will summarize the types of molecular markers that can be used for mapping genes and strategies for mapping monogenic or polygenic disease resistance.

APPLICATION OF MOLECULAR MARKERS

The advancement of the molecular biotechnology opens new vistas to fastened the breeding program of vegetable crops by using a various molecular marker in the different methods of breeding and their steps for enhancing of the improvement program.

Genetic Linkage Maps

It is a graphical representation of an array of loci. Segregating populations, including F_2 generations, backcross progeny, recombinant inbred lines, etc. to be used to study recombination between markers (Lefort-

Buson *et al*., 1990). Using the molecular technique, the selection of plant material depends on the biology of the species and the objectives of the study. Various working on different vegetable crops and other crops also, they developed molecular maps for several crops, including tomato (Tanksley *et al*., 1992) and rice (Causse *et al*., 1994).

Assessment of Genetic Diversity

Diversity studies using molecular markers are now common practice, several workers using this technique in different vegetable crops. Dominant markers like RAPD used for the analysis of pepper breeding lines (Heras *et al*., 1996) revealed very narrow genetic base with more than 50% of the DNA bands being common among all the lines. Villand *et al*. (1998) reported that in an assessment of the world collections of tomato and found South American accessions to have greater diversity than old world accessions. Shim and Jorgensen (2000) also carried out AFLP analysis in diversity studies between wild and cultivated carrots and found that the old varieties released during 1974-1976 and newly developed F_1 hybrid varieties. Archak *et al*. (2002) using RAPD markers in tomato for the same purpose and, found old introductions and locally developed varieties of 1970s exhibiting genetically differed with those who released in 1990s.

Simple sequence repeats (SSR) and sequence related amplified polymorphism markers were used by Ruiz and Martinez (2005) to study the genetic variability of some traditional tomato cultivars of Spain. In this study, it was revealed that the Mexican cultivar Zapotec, a breeding line and virus resistant commercial hybrid "Anastasia" were found genetically most distant of all the cultivar. Muminoric *et al*. (2005) used 12 AFLP and 10 inter-simple sequence repeat (ISSR) primers to estimate genetic diversity in 68 varieties of cultivated radish. According to him substantial level of genetic variability in germplasm of cultivated radish and within cultivated material, black radish and French breakfast radish types formed a separate cluster. In another study, AFLP marker analysis detected a greater genetic variability among American than among Spanish accessions of *Cucurbita maxima* (Ferriot *et al*., 2004). Levi and Thomas (2004) identified 80.2-97.8% genetic similarity among hair loom cultivars of watermelon using ISSR and AFLP markers and they concluded that ISSRs and AFLPs are highly effective in differentiating among watermelon cultivars of elite lines with limited genetic diversity revealed by RAPD markers. Ansari and Singh (2013; 2014) also reported that RAPD and SSR markers are effective in differentiating among the genotypes of *Solanum aethiopicum* and *Solanum melongena*.

Gene Tagging

Gene tagging is a pre-requisite for MAS and map based gene cloning. Tagging of gene in important vegetable crops has been made *viz*., in tomato TMV resistance *Tm-2* locus, nematode resistance, *Mi* gene, *Fusarium oxysporum* resistance gene, and powdery mildew resistance gene, etc. Huang *et al*. (2000) also make possible to tag powdery mildew resistance gene *ol-1* on chromosome 6 of tomato using RAPD and SCAR markers.

DNA Fingerprinting for Varietals Identification

This is an important tool to detect and identify any genotype of crop plants as well as whole living organism. A large number of molecular marker has been used today for DNA fingerprinting of cultivars and breeding lines in a number of vegetable crops *viz*., tomato (Kaemmer *et al*., 1995), beans (Hamann *et al*., 1995) pepper (Prince *et al*., 1995), and potato (McGregor *et al*., 2000). This technology has a great potential for enhancing purity assessment in hybrids also. Genetic purity can also be detected using this technique as reported by Mongkolporn *et al*. (2004) F_1 Chilli hybrids was determined using two molecular techniques RAPD and ISSR. They found that RAPD analysis successfully detected all three F_1 hybridity whereas; ISSR detected only two due to the RAPD marker system producing a greater number of markers than the ISSR system.

Identification of Cultivar

Identification of cultivars is essential today to prevent infringement and duplication of the same genotype in the germplasm conservation and registration. Now in several vegetable crops microsatellites have been developed to enable highly reliable identification of cultivars like tomato, pepper, potato, alliums, cucurbits, lettuce, and spinach. Comparative assessment of five different DNA fingerprinting techniques carried out in tetraploid potato genotype revealed by AFLP to have the highest discrimination power followed in decreasing order by multilocus SSR, RAPD, ASSR, and single locus SSR. In pepper, Gaikwad *et al*. (2001) also found ISSR markers was the most efficient in detecting polymorphism. However, due to very high number of markers generated per assay by AFLP, the marker index of AFLP markers was prominently higher than that of ISSR and RAPD. In another study, Broun *et al*. (1992) identified two telomeric tendemly repeated sequences (7bp) and a closely linked 162 bp subtelomeric repeats in tomato and they accounted for 2% of the total chromosomal DNA. These sequences have a very high mutation rate of 2% in each generation. They have been

shown to be extremely useful for distinguishing and very similar to tomato and melon varieties.

Monogenic Disease Resistance

Development of resistant varieties against important insect-pest and diseases of vegetable crops is the first step to fight against infestation of insect-pest and diseases, which may reduce the use of chemical pesticide and beneficial for the eco-friendly management of insect-pest and diseases of vegetable crops. Use of resistance varieties are also helps in the organic farming or cultivation of vegetable crops. Molecular markers are now using in the resistant breeding program. MAS is based on the concept that it is possible to infer the presence of a gene from the presence of a marker if a narrow linkage has been established between them. This technique has been utilizing in the breeding program to enhance and rapid selection in the early generation. The likelihood of detecting a marker linked to a disease resistance gene is inversely proportional to the genetic distance between the marker and the gene. For a better estimation, the genetic distance between the marker and the gene must be calculated from a large population or better from several crosses, in light of this concept, the genetic distances may greatly vary between crosses (Messeguer *et al*., 1991). Linkages have been frequently observed between markers and monogenic disease resistance by mapping on a genetic linkage map. RFLP or RAPD are the most wide used marker techniques for this strategy.

Polygenic Disease Resistance

Almost all complex disease resistances (i.e., quantitatively expressed) are assumed to be under oligogenic or polygenic control (Mather and Jink, 1971) and/or influenced by the environmental factors too. To solve this problem quantitative trait loci, or quantitative trait locus (QTLs) (Geldermann, 1975) are considered to identify chromosome sites at which genes that have effect on quantitative traits can be located. The quantitative nature of resistance against certain biotic and abiotic stresses would result from the simultaneous and independent allelic variation of such genes involved are influenced by the effect of environment (East, 1916; Yule, 1906). The search for linkages between molecular markers and QTLs of particular quantitative trait is based on this hypothesis.

Detection of QTLs

The detection of linkages between markers and QTLs are the important objective of the breeders engaged in the resistance breeding of crop plants; it can be performed using various statistical methods. The statistical approach using the analysis of variance estimates to fulfill this goal and the degree of association between a genotypic marker (an allelic form) and a phenotypic trait which may also be influenced by various environmental factors. Resulting this, the phonotypical values are the dependent variables and the genotypic markers correspond to the treatment or the factor (source of variation). Analysis of variance models of increasing complexity provide accurate information on the genetic basis of the resistance in the crop plants, for instance, the effect of individual markers (one-way ANOVA), the effect of pairs of markers in the factors of two-way analysis (epistasis by two-way ANOVA) (Lefebvre, 1993). The interval mapping approach (Lander and Botstein, 1989) helps to consider linkages between markers. Using the maximum likelihood equation, the method provides an estimate and also to expressed as limit of detection (LOD) score of the likelihood of the presence of a QTL for regular intervals throughout the genome based on flanking marker information which useful in the genomic study as well as for the sequencing technique of the genome of an organism. The LOD scores actually depend on the localization of the QTL with respect to the flanking markers and the magnitude of its effect; it is also on the probability that there is a QTL present in the chromosome. When examining the curves representing LOD, this method would be very powerful because it accounts for recombination rates of different markers. To use this method, it requires the markers to have been mapped and the trait that have to a Gaussian distribution, a condition although which is not always satisfied in the study of disease resistance genes (semi-quantitative data), ANOVA and interval mapping are the most currently used methods for this technique. Since disease resistance is to be assessed with ordinal scales and data do not always show a normal distribution, concern researchers have been testing putative QTLs with non-parametric statistical tests here (Young and Tanksley, 1989). In another way using maximum likelihood, mean squares, linear, and multiple regressions, have been described for another method (Rodolphe and Lefort, 1993). With the help of different molecular markers, polygenic disease resistance can be partitioned, and individual effects can be examined (components of resistance). Results of genetic studies of complex interactions have been reported and first report on insect resistance in tomato (Nienhuis *et al.,* 1987) and then quantitative resistance to pathogenic fungi and bacteria and nematodes also. In addition, QTL mapping could be useful for identifying loci involved in quantitative components of resistance to viral infections in crop plants and rate of its multiplication as well as its movement in

the host and disease progression. New genes for partial resistance against this problem might be identified by using the technique.

Strategies for Targeted Mapping

Now today it is possible to identify markers for disease resistance genes directly without drawing a genetic linkage map, as drawing of genetic linkage map is a time-consuming procedure. The direct use of molecular markers is essentially limited to monogenic traits only since it consists of identifying a particular genomic region coding for the trait. Studies using aneuploid lines to identify the chromosomes or chromosome arms that carry disease resistance genes and near isogenic lines or bulk segregant analysis to identify markers located near disease resistance genes are the suitable examples for this aspect (Lefebvre, 1993).

DISCUSSION AND CONCLUSIONS

Now open a new perspective for plant breeders by molecular mapping of resistance in crop plants. Understanding of the mechanisms of monogenic and polygenic disease resistances is progressing. In some cases, markers linked to disease resistance have been using for marker-assisted selection (MAS) programs, which are eliminating the need for traditional disease testing procedures. In his study, Melchinger (1990) optimized a design for retaining the minimum number of individuals in each generation, relying on the recombination rate between the target gene and 1 or 2 markers. For a major resistance gene, marker based recurrent backcross programs are using frequently (Young *et al*., 1993). MAS can be useful to "pyramiding" resistance genes in a genotype of desirable type. Future application of MAS will be in quantitative resistance loci. In case of small number of QTLs are involved, the technique becomes similar to that used to select qualitative traits. For more loci of segregating for a trait larger number of individuals must be characterized to have a high probability of recovering the favorable set of marker alleles at all the interesting loci. If a particular trait is controlled by a large number of QTLs with small effects, the probability of identifying markers linked to all the QTL is low and moreover there is a high probability of finding a single false QTL, which representing the resultant effect of many small effect genes dispersed to be difficult for making selection. A selection index comprising molecular marker information and phenotypic scores both will produce more progress from selection than marker selection or phenotypic selection alone unless heritability of the trait is 1 (Lande and Thompson, 1990). In a separate study Dudley (1993) reviewed methods and results concerning MAS for traits controlled by a large number of QTLs and discussed useful methods of combining data from different markers with different traits. The potential of MAS in quantitative genetics still remains unclear because it depends on the cost involved and the complexity of the techniques. There are still technical limitations for generalizing their use everywhere (DNA extraction, southern transfer, and hybridization for RFLP). With the development of the PCR-based techniques, MAS is greatly simplified (Lefebvre, 1993).

There are several traditional and modern breeding tools and techniques that can be utilized for crop improvement in organic vegetables despite the ban on genetically modified organisms. The controlled crosses between individuals produce desirable genetic variation to be recombined and transferred to next progeny through natural process. MAS can also be employed as an effective tool to facilitate selection of progeny in an early generation who have desired trait(s) resulting speeding up of the selection procedure in the breeding program. This technique is useful for the introgression of resistance genes into new backgrounds and the efficient selection of many resistance genes for pyramiding into a single genotype. Unfortunately, molecular markers are currently unavailable for several important traits controlled by many genes or polygenes.

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