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Cytogeneticist's insight about molecular markers and their role in crop improvement

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Abstract

The productivity of domestic crop plants has evolved through the collective efforts of scientists since the dawn of agriculture. From a historical perspective, improved crop yields have been influenced perhaps more by genetic improvement than by any other single factor. Despite the breeding progress already achieved, additional gains in agricultural productivity are demanded at an ever-faster pace by population growth and by changes in agricultural practices, biotic and abiotic environments and consumer preferences. Feeding 8 billion by 2025 AD and 10 billion by 2050 AD will be the sole task for agricultural scientists and the policy makers for coming 50 years. Of the several routes that can result into greater crop production and increased world food supply, increasing yield per hectare per crop through increased genetic yield potential will be the most important route. Crop breeders have envisioned and are following several approaches for sustainable improvement of crop productivity. Of these empirical selection; analytical breeding; ideotype breeding and alien introgression are some of the approaches which crop breeders are following for further improving the crop productivity.

Key words: Cytogeneticist's insight, molecular markers, and crop improvement

Introduction:

The productivity of domestic crop plants has evolved through the collective efforts of plant scientists since the dawn of agriculture and represents mankind's greatest achievements. From a historical perspective, improved crop yields have been influenced perhaps more by genetic improvement than by any other single factor (Fehr, 1984). Despite the breeding progress already achieved, additional gains in agricultural productivity are demanded at an ever-faster pace by population growth and by changes in agricultural practices, biotic and abiotic environments and consumer preferences.

Feeding 8 billion by 2025 AD and 10 billion by 2050 AD will be the sole task for agricultural scientists and the policy makers for coming 50 years. Of the several routes that can result into greater crop production and increased world food supply, increasing yield per hectare per crop through increased genetic yield potential will be the most important route (Evans, 1999). Crop breeders have envisioned and are following several approaches for sustainable improvement of crop productivity. Of this empirical selection (Evans and Fischer, 1999); analytical breeding (Edmeades *et al*., 1997); ideotype breeding (Khush and Peng, 1996) and alien introgression (Tanksley and McCouch 1997) are some of the approaches which crop breeders are following for further improving the crop productivity. Here we will discuss in brief (under the heads presented below) about molecular markers and the ways these can be used for further improving and sustaining crop productivity. For more comprehensive reviews readers may refer to Tanksley *et al*. (1989); Paterson *et al*. (1991); Brar and Dhaliwal (1997); Mohan *et al*. (1997) and Joshi *et al*. (1999).

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I. Types of molecular markers

Several types of molecular markers which have been developed and used in plants are restriction fragment length polymorphism (RFLP), sequence tagged sites (STS), expressed sequence tags (ESTs), simple sequence repeats (SSRs) or micro satellites, randomly amplified polymorphic DNA (RAPDs), sequence characterized amplified regions (SCARs) and amplified fragment length polymorphic (AFLP) markers. In addition several other variations of these markers have been developed (see Joshi *et al*., 1999).

Restriction fragment length polymorphism (RFLP): These are single or low copy DNA fragments and are simply inherited. These probes could be genomic clones, cDNA clones, or even cloned genes. The RFLP markers show co-dominance and are highly reliable in linkage analysis and breeding. Their detection is based on radiolabeling, requires large quantities of DNA, are labour intensive and relatively expensive and hazardous. Hence, their large-scale use in practical plant breeding may be restricted.

Sequence tagged sites (STS): The RFLP probes, linked to desirable traits can be converted to polymerase chain reaction (PCR) based markers. In this the RFLP probes are end sequenced and complementary primers are synthesized. These primers (generally 20 mers) are then used for amplifying specific genomic sequences using PCR. For example, STS markers have been developed for RFLP markers linked with bacterial blight resistance genes *xa5, xa13* and *Xa21,* powdery mildew and stem rust resistance gene in barley etc. One major limitation of these markers is the reduced polymorphism than the corresponding RFLP marker.

Expressed sequence tags (EST): These markers are developed by end sequencing of random cDNA clones. The cDNA markers are first mapped as RFLP markers and then partially sequenced to convert them into PCR based markers. Thus, these are like STS markers. These can be used for synteny mapping and cloning of specific genes. Most of these could be functional genes. Large number of EST markers has been identified in rice (more than 1450) (Hanushima *et al*., 1998) and *Arabidopsis*.

Simple sequence repeats (SSRs): Also called as micro satellites, these are short tandem repeats dispersed throughout the genome. These are generally di-to-tetra-nucleotide repeats and are hyper variable. These are flanked with unique sequences, which are highly conserved. The flanking unique sequences are analyzed and their complementary primers synthesized. These can thus be assayed with PCR and act as co dominant markers. Referred to as simple sequence length polymorphism (SSLP), allelic differences are usually as a result of variable number of repeat units. These are highly polymorphic. Their major limitation is the cost involved in its development. Micro satellite markers have been developed and incorporated in already existing RFLP linkage maps in crops like rice (McCouch *et al*., 1997), wheat (Roders *et al*., 1998) and several other monocot and dicot species (Mohan *et al*., 1997).

Randomly amplified polymorphic DNAs (RAPDs): The technique was originally developed by Williams *et al*. (1990). In this arbitrary decamer sequences are used as primers for amplification. These markers are dominant markers because the polymorphism is due to presence or absence of a particular amplified fragment. One major advantage of these markers is that this does not need any prior sequence information. These markers have been used for constructing linkage maps in several species and also for tagging genes of economic importance. One major limitation of these markers is lack of repeatability in certain cases.

Sequence characterized amplified regions (SCARs): These markers overcome the limitation of RAPDs. In this, the RAPD fragments that are linked to a gene of interest are cloned and their termini sequenced. Based on the terminal sequences, longer primers (20 mers) are designed. These SCAR primers lead to a more specific amplification of particular locus. These are similar to STS markers in construction and application. The presence or absence of the band indicates variation in sequences. The SCAR markers thus are dominant markers. These however, can be converted to co dominant markers in certain cases by digesting the amplified fragment with tetra cutting restriction enzymes. The RAPD primers linked to important genes have been converted to SCAR markers in several cases (see Joshi *et al*., 1998).

Amplified fragment length polymorphism (AFLP): This technique was developed in Vos *et al*. (1995). In this technique restriction fragments generated by a frequent (4 base) and a rare (6 base) cutter are anchored with oligonucleotide adapters of a few oligonucleotide bases. This method generates a large number of restriction fragments facilitating the detection of polymorphism. Choosing different base numbers and composition of nucleotides in adapters can control the number of DNA fragments, which are amplified. This technique is more reliable since stringent reaction conditions are used for primer annealing. This technique thus shows an ingenious combination of RFLP and PCR techniques and is extremely useful in detection of polymorphism even between closely related genotypes. AFLP maps have been constructed in several species and integrated into already existing RFLP maps (for example in tomato) (Haanstra *et al.*, 1999).

The various types of molecular markers presented above have specific features that direct their use. A comparison of features of these markers is presented in Table 1.

II. Development of saturated maps

In the past genetic maps were based mainly on morphological and isozyme markers. But these markers are limited and are influenced by environment and developmental stage. Molecular markers on the other hand are large in number and are not influenced by the environment and developmental stage. Saturated linkage maps are a pre-requisite for gene tagging, marker assisted selection and map based gene cloning. Saturated linkage maps have been developed in several crop plants like maize, rice, tomato, wheat, potato, barley, cotton, *Brassica* etc. For example, rice has more than 2000 markers (Harushima *et al.*, 1998), tomato has more than 1500 markers (Haanstra *et al.*, 1999), D-genome of wheat has more than 550 markers (Boyko *et al.*, 1999) mapped on these. In rice centromere positions in all the 12 linkage groups have been defined (Singh *et al.*, 1996). The markers used for developing saturated maps include RFLPs, RAPDs, micro satellites and AFLPs.

III. DNA fingerprinting for varietal identification

Comprehensive review on the need for fingerprinting of crop varieties has been presented by Smith and Smith (1992). DNA fingerprinting can be used for varietal identification as well as for ascertaining variability in the germplasm. Although any type of marker can be used but RAPDs, micro satellites and AFLPs are the markers of choice for the purpose because all these are PCR based and do not require any prior information on nucleotides. The fingerprinting information is useful for quantification of genetic diversity, characterization of accessions in plant germplasm collections and for protection of proprietary germplasm especially the cms lines. These markers have been used to differentiate even closely related cultivars (Melchinger *et al.*, 1991). Paull *et al.* (1998) analyzed 124 Australian major wheat varieties and important lines. They were able to distinguish even closely related lines and classified these into four groups. One of the most recent application of molecular markers has been shown in sex identification of dioecious plants (Parasnis *et al*., 1999). Here, micro satellite markers $(GATA)_4$ are found to reveal sex-specific differences. This can be used as a diagnostic marker for identifying male and female plants, right at seedling stage.

- Sequence information required
- Hav, average heterozygosity. An average for the probability that two alleles taken at random can be distinguished.
- N/A, data not available in the reference cited.
- Effective multiplex ratio is the number of polymorphic loci analyzed per experiment in the germplasm tested.
- Marker index is the product of the average expected heterozygosity and the effective multiplex ratio.
- Between laboratories, influenced by Taq polymerase and thermocycler
- Also savings of time compared to RFLP
- Cost of initial sequencing high
- Became easier with practice
- Some technical problems associated with silver staining.

IV. Phylogenetic and evolutionary studies

An important use of genetic markers has been to attempt to discern evolutionary relationships within and between species, genera or larger taxonomic groupings. Such studies involve studying similarities and differences among taxa using numerous genetic markers (Paterson *et al*., 1992). Although phylogenetic trees have previously been established for many species on the basis of visible and isozyme markers and chromosome homology, the DNA markers have recently added to length and breadth of phylogenetic information available for a number of species.

A large number of such studies have been made in most of the crop plants. Enlisting all these is not possible here. Two examples in rice, we will like to cite here to make clear the use of these techniques. Two lines of rice Azucena and PR 304 were classified as indicas using morphological characters while these behaved as japonicas in crossing studies. When these lines were analyzed using molecular markers, these grouped with japonica. Similarly, the genomic constitution of wild species *Oryza redleyi* and *O.granulata* were not known, as these were not crossable with all the species whose genomes are known. Using genomic *in situ* hybridization (GIS), Aggarwal *et al*. (1997) were able to elucidate the genomes of these species and designated these as GG for *O. granulata* and HHJJ for *O. indleyi*. Although all the types of molecular markers described above can be used for establishing phylogenetic and evolutionary relationships, but RAPDs and RFLPs have been used more frequently so far.

V. Molecular markers and heterosis breeding

One of the earliest conceived idea about the use of molecular markers was its use in heterosis breeding. Earlier results of Lee *et al*. (1989) in corn suggested that RFLP analysis may provide an alternative to field-testing when attempting to assign maize inbreds to heterotic groups. Since then several attempts were made to correlate heterosis with variability at molecular level. Melchinger *et al*. (1991) analyzed 32 maize inbred lines for molecular markers diversity. It was concluded that molecular markers were useful for assigning maize inbreds to established heterotic groups and investigating relationships among the inbred lines. However, degree of heterozygosity at RFLP loci was not associated with heterosis for yield for crosses among unrelated lines. Zhang *et al*. (1995) on the other hand observed a high correlation between specific heterozygosity and mid parent heterosis in rice.

A second aspect of heterosis breeding relates to genetic basis of heterosis. Two basic hypotheses i.e. dominance and over dominance were proposed at the beginning of this century. The controversy still continues at the end of this century (Bains *et al*. 1999). Attempts were made to resolve this controversy using molecular markers. Stuber *et al*. (1992) mapped QTL's contributing to heterosis in the cross between the elite maize inbred lines B73 and Mo17. The QTL's detected for grain yield showed that heterozygotes had higher phenotypic value than the respective homozygotes, thus, suggesting over dominance (or pseudo over dominance) as the basis of heterosis. Contrary to this, Xio *et al*. (1995) mapped QTL's for heterosis in one of the highest yielding indica x japonica hybrids and proposed dominance as the major cause of heterosis in rice.

VI. Gene tagging

Gene tagging refers to mapping of genes of economic importance close to known markers. Thus, a molecular marker very closely linked to a gene can act as a `tag' that can be used for indirect selection of gene in breeding programmes. With the construction of molecular map, especially the RFLP maps, several genes of economic importance like disease resistance, stress tolerance, insect resistance, fertility restoration genes, yield attributing traits etc. have been tagged (Table 2). Gene tagging is a pre-requisite for marker-assisted selection and map based gene cloning. In addition, gene mapping is throwing more light on evolution of several genes. For example, in *Brassica napus*, two cms sources, *nap* and *pol* are restored by two different restorers, *Rfn* and *Rfp*. Mapping studies showed that both the genes map to same chromosomal region (Li *et al*., 1998).

VII. Marker assisted selection

Plant breeders have relied heavily on generating new gene combinations and selecting these new gene combinations empirically. The response in the improvement had been tremendous so far and its efficiency can further be improved by marker-assisted selection. The essential requirements for marker-assisted selection in a plant-breeding programme are:

a) Marker(s) should co segregate or be closely linked (less than 1cM) with the desired trait. An efficient means of screening large populations for molecular marker(s) should be available. PCR based technique to some extent fulfill this.

c) The screening technique should have high reproducibility across laboratories, be economical to use and should be user friendly.

Marker assisted selection can be practiced more efficiently for characters whose phenotypic selection is difficult. For example, transferring a fertility restorer gene from one line to another line through backcrossing needs test crossing before subsequent backcrossing. If such genes are tagged with molecular markers, desirable plants with fertility restorer gene (in heterozygous condition), can be identified and backcrossed. Similarly, screening for abiotic stresses is very difficult. If desirable genes conferring tolerance to abiotic stresses are tagged, these can be selected easily in segregating generations. Also genetic markers can be assayed in non-target areas such as growth chamber, green houses or off-season nurseries, thus permitting more rapid progress.

The efficiencies of scale and time accorded by DNA markers are valuable in breeding most species but are of special value in breeding species with large stature or long generation time, such as orchard or forest trees, where fewer individuals might save hectares and fewer generations may save decades (Paterson *et al.*, 1992).

Marker assisted selection (MAS) is being more efficiently used in the following areas:

i. Gene pyramiding.

ii. Marker assisted alien introgression.

iii. Simultaneous identification and pyramiding of QTL's from primitive cultivars and alien species.

i) Gene pyramiding: MAS has been incorporated in several breeding programmes to pyramid several disease and insect resistance genes in a single line for increasing durability of resistance. In conventional breeding procedures, it is generally not possible to combine several genes in one line due to `Vertifolia effect' i.e. a dominant gene showing resistance to several races masks the effect of other genes, which are showing resistance to one or more races. Such genes whose effect is masked cannot be selected precisely using conventional techniques. Similarly, combining a dominant and a recessive gene may not be possible. However, it is possible under both the situations using MAS. An excellent example in this regard comes from rice. The bacterial blight genes *xa5, xa13* and *Xa21* have been tagged with STS markers. At PAU, Ludhiana, these three genes have been pyramided in most popular rice variety PR 106. The pyramided lines of PR 106 with all the three genes are showing complete resistance to bacterial blight (Singh *et al.* in preparation). Several bacterial blight genes have been pyramided in indica variety IR 24 (Huang *et al.*, 1997) and in a japonica variety also (Yoshimura *et al.*, 1995). Similar works have been initiated in other crops also.

ii. Marker assisted alien introgression: Alien introgression has been one of the major activity of most plant breeding programmes. Several alien genes like *Lr*9, *Lr*19, *Lr*21, *Lr*24, *Lr*29 conferring resistance to leaf rust and many others have been transferred in wheat from wild species. Similarly, in tomato genes like *Mi* (nematode resistance), Tm2a (TMV resistance) have been transferred from wild species (Kaloo and Chaudhary, 1992). Despite effectiveness of alien introgressed genes against several diseases and insect pests, all these genes have not found their way in commercial lines mainly due to linkage drag i.e., accompanying of undesirable traits (Friebe *et al*., 1996). Stephens (1961) eloquently summarized the hazards of interspecific breeding as:

When the chromosomes of different species are sufficiently alike to recombine more or less freely, their recombinant products are likely to be greatly inferior to the parental combinations. At present, there is no way of controlling this total disruption of the parental genotypes which result from segregation".

Genetic markers provide a means of controlling the degree of disruption of parental genotype. Selection in the first backcross generation using molecular markers, one can restore most of the recurrent parent genotype while retaining the desirable genes from the wild parent. This can be done by selecting against the markers from the wild parent outside the region carrying target genes (Young and Tanksley, 1988). Using this technique Young and Tanksley (1989) selected for Tm 2a gene with minimum backcrosses thus saving time and eliminating linkage drag.

In addition to precise alien gene transfer, molecular markers can be used for monitoring alien gene transfers and understanding the mechanism of gene transfer. Jena *et al*. (1992) used RFLP markers for detecting introgression in interspecific backcross derived lines of the cross *O. sativa* (AA) x *O. officinalis* (CC). They detected introgression from 11 of the 12 *O. officinalis* chromosomes. The introgressed segments were small and most of these showed reciprocal replacement of alleles of cultivated rice with the alleles from wild species i.e. the gene transfer occurred due to recombination. Although gene transfer was restricted but this did not corroborate with the level of pairing observed during meiosis-1 at diakinesis or metaphase stages. This implies that level of pairing observed at diakinesis or Metaphase-I may not be an exact reflection of what is happening at zygotene stage. Brar *et al*. (1996) detected introgression for 1 or 2 RFLP markers from *O. brachyantha* (FF) and *O. granulata* (GG) into rice indicating the possibility of introgressing useful genes even from distantly related genomes into cultivated rice.

iii. Simultaneous identification and pyramiding of QTL's from primitive cultivars and alien species: Most of the characters of economic importance are governed by quantitative genes. Conventional breeding has relied exclusively on empirical selection of these quantitative trait loci (QTL's) in cultivated species. Until recently, there was no way out for dissecting and directed manipulation of such loci. Molecular markers, however, have made it possible to dissect individual QTL's, both in cultivated and alien germplasm and further directed selection of these QTL's. First decade of the new millennium should see the large-scale application of this technique in crop improvement programme.

Tanksley and Nelson (1996) proposed advanced backcross QTL (AB-QTL) analysis method of combining QTL analysis with variety development. This method aims at simultaneous discovery and transfer of valuable QTL alleles from unadapted donor lines (viz., land races and wild species) into established elite lines. The steps involved in AB-QTL analysis are:

- Crossing a recipient (elite line) with a donor (land race, wild species etc.) line.
- The F_1 is backcrossed to recipient line to generate sufficient BC_1 seed (100-200 plants).
- phenotypically selected desirable BC_1 plants are crossed to the recipient parent to generate BC_2 plants.
- The BC_2 plants are subjected to RFLP analysis and are selfed to generate BC_2F_2 families. Also some desirable BC_2 plants are backcrossed to generate BC_3 plants.
- The BC_2F_2 families are evaluated and analyzed for QTL's. Several QTL-near isogenic lines (QTL-NILs) are isolated by whole genome selection.
- The different QTL-NILs possessing different QTL's for a character can be crossed and all the desirable QTL's can be pyramided in a single line.

Using this technique, several desirable QTL's have been identified and transferred into crop plants. For example, in tomato, QTL's for several characters have transferred from *Lycopersicon pimpinellifolium* (Tanksley *et al.*, 1996) and *L. peruvianum* (Fullan *et al.*, 1997). Similarly, QTL's for yield and yield improving traits have been identified and transferred into rice *O. sativa* from its wild relative *O. rufipogon* (Xiao *et al.*, 1996; 1998). Recently, an international project on molecular breeding of rice has been initiated in rice under the leadership of Dr.G.S.Khush (G.S.Khush, pers.comm.). This project aims at identification of QTL’s from land races across the globe and pyramiding the desirable QTL’s into elite lines. More than 35 centres throughout Asia are participating in this project.

VIII. Orthologous gene mapping

Molecular markers are being used extensively for studying the divergence and evolution of crop plants. "Comparative mapping" is the name given to this approach. In this marker clones especially cDNA clones of one crop plant are being mapped onto the linkage maps of other crops. This approach of comparative mapping is useful in several ways:

i. More saturated maps can be generated by mapping marker clones of one crop onto the linkage map of other crops.

ii. By cross mapping divergence and evolutionary history of various crop plants can be revealed.

iii. It can make gene cloning from complex organisms comparatively easier.

The first comparative map in plants was generated by the group of S.D.Tanksley in tomato, pepper and potato genomes (Tanksley *et al.* 1988). Since then comparative maps have been generated in several crops especially in grasses (Gale and Devos, 1998). By cross hybridization of RFLP markers, the conserved gene order or synteny has been established in several families. The rice genome, for example, has been divided into 19 `genomic blocks' or `Lego blocks'. Joining these `Lego blocks' in different combinations will result into genomes of sorghum, sugarcane, foxtail, maize and wheat (Moore *et al*., 1995).

Microscopic synteny is utilized for estimation of correspondence of loci among these cereal crops. Rice because of having small genome size, can be used as a model crop among grass species for gene isolation. For example, synteny has been established between the Ph1 locus of wheat (chromosome 5BL) and a region of chromosome 9 of rice (Foote *et al*., 1997). Similarly, through comparative mapping, Kiliani *et al.* (1995) have identified rice genomic region that encompasses barley stem rust resistance gene *Rpg-1*, a disease to which rice is completely insensitive. The loci for comparative mapping include not only the major genes but QTL’s also (Paterson *et al*., 1995).

Effectiveness of orthologous genes in different backgrounds has been demonstrated recently using gibberelic acid insensitive (*gai*) genes conferring dwarfness in crop plants (Peng *et al*., 1999). A *gai* gene construct from *Arabidopsis* was introduced into rice variety Basmati 370. The transformed line showed reduced height. This demonstrates effectiveness of orthologous genes in different backgrounds. Using this construct, DNA sequences complementary to wheat dwarfing gene *Rht B-1* and maize dwarfing gene *d8* were isolated and their amino acid sequences compared. This is the first example to our knowledge that demonstrates cloning of a gene using orthologous gene as a probe.

IX. Map-based gene cloning

It refers to the isolation of a gene corresponding to a target trait using molecular maps. Saturated molecular maps offer opportunity for isolating genes whose biochemical products are not known. Map-based cloning consists of four major steps:

a). Development of a high-resolution molecular linkage maps in the region of interest.

b). Physical mapping of the region of interest. This can be achieved through generation of yeast artificial chromosome (YAC) or Bacterial artificial chromosome (BAC) contigs.

c). Identification of appropriate YAC or BAC clones for isolating putative clones harbouring the gene of interest.

d). Verification through transformation that the target gene is isolated.

The strategy of map-based cloning is well reviewed by Tanksley *et al*. (1995). Development of saturated molecular linkage maps, physical maps such as BACs or YACs and accelerated DNA sequencing techniques has greatly enhanced the prospects of map-based gene cloning. Several genes, especially disease resistance genes, have been cloned using map-based gene cloning strategy. Martin *et al*. (1992) were the first to clone a disease resistance gene *Pto* in tomato using map-based cloning. Since then several disease resistance genes conferring resistance to fungi, bacteria, viruses and nematodes have been cloned (Table 3). Cloning of resistance genes and subsequent comparison of their gene products has revealed striking similarities among these genes. Structural similarities in disease resistance genes have changed the concept of disease resistance in plants to "disease resistance beyond the resistance genes" (Gopalan and He, 1998). Cloned genes *Xa21* in rice have been transformed into bacterial blight susceptible variety IR and the transgenic line is resistant to bacterial blight (Tu *et al*., 1998).

Last 10-15 years saw major emphasis on development of molecular markers. In the coming decade, the major emphasis definitely will be laid on the use of these markers in crop improvement programmes. Alien introgression coupled with molecular markers, we believe, should bring another quantum jump in next 10-15 years.

Table 1: Comparison of marker techniques commonly used in plant research

Feature	Marker system			
	AFLP	RFLP	RAPD	SSR
DNA required (: g) PCR based Level of polymorphism (Hav[b]) Effective multiplex ratio[d] Marker index (MI[e]) Reproducibility Cost Ease of use	0.5-1.0 Yes 0.11 0.29-0.64 0.32 Medium 50-100 19.2 24.7 6.14 Very high N/A $105[g] Medium Difficult initially	10 No 0.28 N/A 0.41 High N/A 0.25 3.0 0.1 Very high N/A $178 N/A Labour intensive	0.02 Yes N/A[c] 0.33-0.34 0.31 Medium 20-50 1.56 N/A 0.48 Fair[f] 2xAFLP N/A Low Easy	0.05-0.10 Yes[a] N/A 0.47-0.76 0.6 Very high 1.0 1.0 N/A 0.6 Very high N/A N/A High[h] Easy[j]

Source: Rodent and Donini (1999)

Table 2: Selected examples of desired genes in selected crop plants tagged with molecular markers[a]

Crop	Trait/gene[b]
Rice	• Blast resistance genes Pi-2(t) Pi-4(t), Pi-10(t) • Bacterial blight resistance genes *Xa1, Xa2, Xa3, Xa4, xa5, xa13* and *Xa21* • Gall midge resistance genes, *Gm2, Gm4t* • Rice Tungro spherical virus (RTSV) resistance • Brown plant hopper resistance *Bph-1, Bph-10(t)* • White backed plant hopper resistance • Fertility restorer genes *Rf-1, Rf-2, Rf-3* • Wide compatibility locus S-5
Wheat	• Cereal cyst nematode • Leaf rust resistance genes *Lr9, Lr10, Lr19, Lr24* • Stem rust resistance genes *Sr21, Sr33* • Powdery mildew resistance genes *Pm-1, Pm-2, Pm-12* • Russian aphid resistance gene Dn2 • Homeologous pairing inducer gene *Ph1b* • Vernalization locus *Vrn1* • Free threshing locus Q • Awn inhibitor locus B1
Rye	• Fertility restoration gene *Rfg-1*
Maize	• Leaf blight resistance gene *rhm* • Northern corn blight resistance gene *Htn-1* • apomixis locus from *Tripsacum dactyloides*
Pearl millet	• apomixis locus from *Pennisetum squamulatum*
Barley	• Stem rust resistance gene *Rpg 1, rpg 4*
Brassica	• Fertility restorer genes for *Polima* and *Ogura* cytoplasms
Soybean	• Cyst nematode resistance • Resistance to soybean mosaic virus • Linolic acid content to *Fan* locus
Tomato	• TMV resistance, *Tm-2* locus • Nematode resistance, *Mi* gene • *Fusarium oxysporum* resistance gene *I1* and *I2* • Powdery mildew resistance genes • Joint less • Soluble solid content
Potato	• Late blight resistance gene *R1* • Cyst nematode resistance gene *H1*

Sources: Mohan *et al*. (1997), Brar and Dhaliwal (1996).

a. The list is not comprehensive and the references are not included due to limitation of space.

b. Although all the genes included here have been tagged with one or the other marker, but all these may not be amenable for marker-assisted selections as most of these are not tightly linked.

Table 3: Selected examples of gene cloned using map-based gene cloning strategy

Species	Trait	Gene
Arabidopsis	• Resistance to Bacterium *Pseudomonas syringae* • Flowering inducer gene • Resistance to foreign *Personospora parasitica*	*RPM1* *Co* *RPP5*
Rice	• Bacterial blight resistance • Bacterial blight resistance	*Xa1* *Xa21*
Tomato	• Resistance to bacterium *Pseudomonas syringae* • Resistance to insecticide Fenthion • Resistance to fungus *Cladosporium fulvum* • Root knot nematode resistance	*Pto* *Fen* *Cf-9* *Mi*
Tobacco	• Tobacco mosaic virus resistance	*N*
Beet	• Cyst nematode resistance	$Hs1^{Pro1}$

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