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Review

From Markers to Genome Based Breeding in Horticultural Crops: An Overview

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Abstract: Molecular markers, genome sequencing and genome editing are considered as efficient tools to accomplish demands of plant breeders for crop improvement programs. Morphological and biochemical markers have not been extensively used as these are greatly influenced by environmental factors. Different molecular markers and sequencing techniques are routinely used in evaluation of genetic diversity and evolutionary relationship, accurate classification or taxonomy, characterization of germplasm, identification of hybrids and phylogenetic studies. Desired and undesired traits controlled by genes can be identified through different molecular markers technology all over the globe. These molecular markers are well established and have successfully been used for genetic analysis of different plants during last two decades. Recently, advanced techniques of molecular markers have been developed, which provide advance genotypic platform and tends to merge valuable properties of many basic systems. New class of markers also includes little modifications in basic methods to enhance the sensitivity and resolution. Biotechnologists, plant breeders and strong investment are strongly linked for crop improvement purposes. Current review provides detailed description of different markers, genome sequencing and genome editing that have been utilized for different genetic analyses of horticultural crops. Genome editing technologies based on CRISPR/Cas are now successfully applied in horticultural crops. Moreover, current review encourages the use of molecular marker technology for DNA fingerprinting, bar coding, sequencing, re-sequencing QTL mapping, genome association mapping and genome editing. It is need of time that diverse germplasm should be identified with different molecular techniques and further utilized in breeding purposes to achieve higher yielding and resistant cultivars against biotic and abiotic stresses.

Keywords: Genetic analysis; genome sequencing; genome editing; molecular markers; plant breeders

1 Introduction

Information regarding genetic diversity, phylogenetic studies, genome sequencing and genome bar coding through utilization of molecular markers plays an imperative role in characterization of diverse germplasm. Desired traits can be determined and sequenced through specific sequence based markers.

Functional markers are efficient tools for evaluation of disease resistant genes in horticultural crops. Important agronomic and yield traits can be sequenced through QTLs mapping and genome association. Genetic diversity information of inter-population as well as intra-population is helpful for conservation and evolutionary biology [1]. The replacement of various genotypes and introduction of new cultivars are major cause of poor genetic variability. This situation of poor variability is very alarming for future breeding programs [2]. So, broadening of gene pool is necessary for improvement of desired traits in horticultural crops. Germplasm characterization was performed though morphological, physical and biochemical markers since ancient times. Isozymes are first biochemical markers used for identification of germplasm [3]. Morpho-physical and biochemical markers are greatly influenced by climatic conditions. Therefore, use of molecular markers is encouraged because these are not affected by any external impacts. Molecular markers are composed of nucleic acids as well as proteins and used on basis of naturally occurring polymorphism [4].

Markers are morphological traits, biochemical properties and DNA sequences that can be used for identification of cultivars, hybrids, species, genera, or their desired traits. Phenotypic markers used by Gregor Mendel in 19th century are highly influenced by environmental conditions, which provide a pathway toward development of DNA based markers, also known as molecular markers. Classical markers include morphological and biochemical markers [3]. Application of morpho-physical and biochemical markers are now considered as insufficient tools for accurate gene mapping for plant breeding programs. However, physical mapping of plants on the basis of morphological and biochemical markers are playing a significant role in genomic mapping with the help of molecular markers.

In the world, a rapid increase in the knowledge of genome sequences, genome bar coding and genome variability was recorded during last three decades which brought great revolution in genetic analyses of plant species. In Pakistan, different research stations are working on identification and adaptation of germplasm focusing on morphological and biochemical markers. The use of molecular markers is very poor and neglected especially in horticultural crops. However, limited use of molecular markers was noticed in agronomic crops i.e., cotton, wheat, maze and rice. Current study provides the knowledge of complete genome that will play an imperative role in gene discovery and improvement of plant species. Gene discovery through different molecular markers is necessary for development of excellent cultivars with desired traits. Moreover, present study provides the strategies and application of different marker technologies for improvement of genome based breeding in horticultural crops. So, genome sequencing and editing approaches will further increase the potential of existing genomic technology to enable genome based breeding in horticultural crops.

2 Ideal Properties of Molecular Markers

DNA based markers comprised of hybridization based, PCR based and sequence based markers. Further detail regarding the markers classification is presented in Fig. 1. An efficient and appropriate marker system has the following ideal properties for successful evaluation of various plant breeding programs [5].

- Easy to use and low cost.
- Highly reproducible and highly polymorphic in nature.
- Allele's difference must be clearly measured.
- Equal distribution through the whole genome.

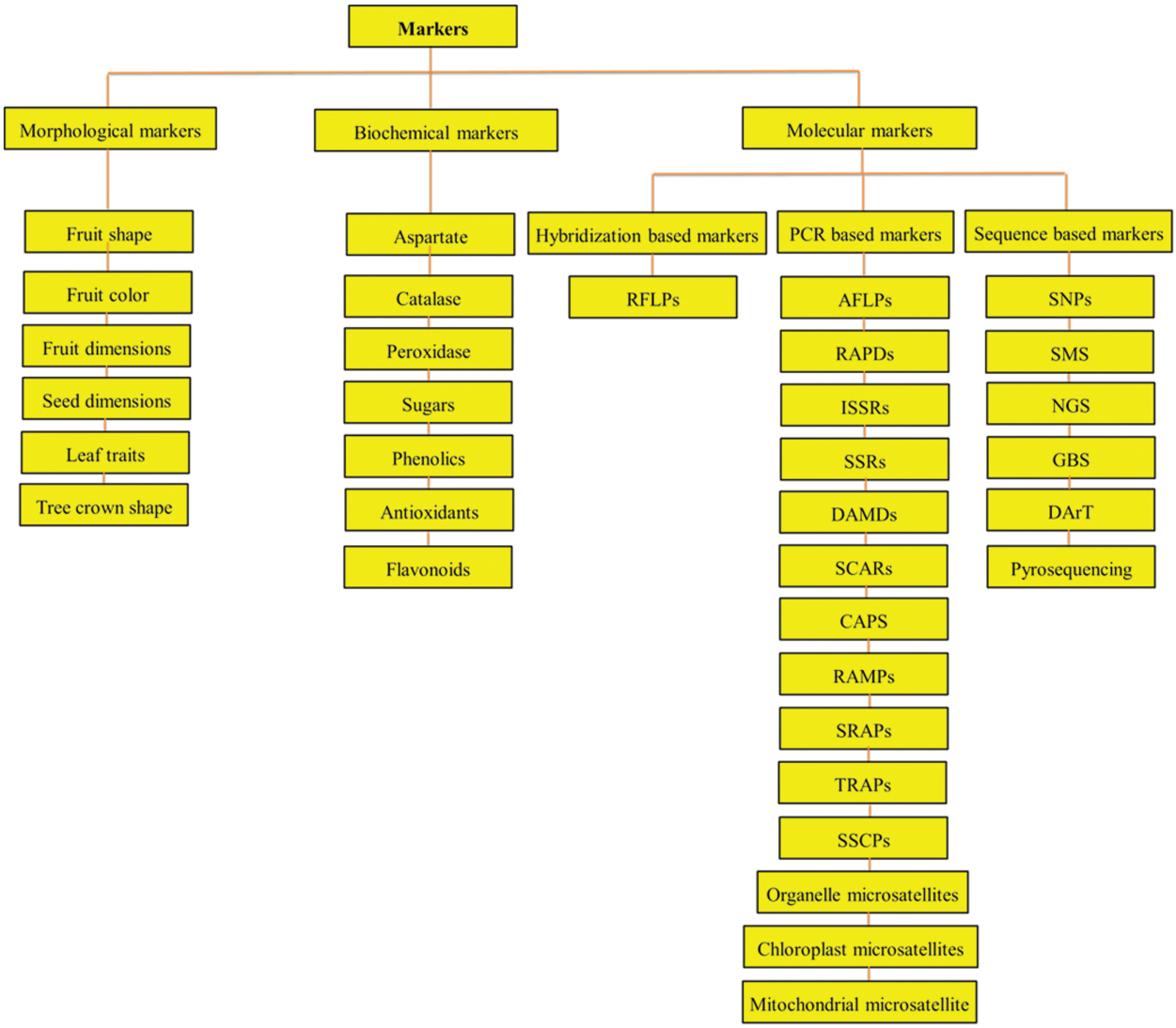

Figure 1: Classification of markers

3 Hybridization Based Markers

Restriction fragment length polymorphism (RFLPs) are the first hybridization based markers that were used for human and plant genome mapping [6]. Mutation can occur at restriction sites in the genome of living organisms. During mutations, the insertions and deletions of base pair at restriction sites may cause variation in restriction fragments size. These differences or variation are the main cause of recognition sites for restriction enzymes. When homologous chromosomes are exposed to restriction enzyme digestion then various restriction products are formed. These restriction products may be identified through different DNA analytical methods. RFLPs can be used for fair genome mapping and disease analysis. These are of co-dominant and locus-specificity nature. The use of RFLPs is very limited because many plant researchers think these require large quantity of high quality DNA, and are very tough to systematize. RFLPs have been used for closely linked taxa [7].

4 PCR Based Markers

Amplified fragment length polymorphism (AFLPs) are also known as selective restriction fragment amplification. Generally, AFLPs are based on the selective PCR amplification of restriction fragments

from a total double-digest of genomic DNA. AFLPs have ability to anneal their target sequences and few nucleotides adjacent to the restriction sites. Two restriction enzymes (a rare cutter and a frequent cutter) are used to digest the genomic DNA. So, fragments will be amplified which have been cut by the frequent cutter and rare cutter. AFLPs are highly reproducible across laboratories, knowledge of prior sequence information is not required and primers are universal which can be used for many species. AFLPs application requires excellent quality of DNA for complete digestion of restriction enzyme and low level of polymorphic information content [8].

Random amplified polymorphic DNA (RAPDs) are dominant markers. Total genomic DNA of an individual is amplified by PCR reaction with a single, short and random primer. Agarose gel electrophoresis system is used to separate the amplified products. Polymorphism produced from a mutation can become visible with the presence or absence of bands during gel electrophoresis. RAPDs are easy to use, require small amount of DNA and produce high level of polymorphism. During primers designing, hybridization, DNA probes and sequence information are not required. The primers of RAPDs are non-specific for species and are universal for all crops. Horizontal or vertical gel electrophoresis is used to separate the amplified products. The applications of RAPDs are limited due to low level of reproducibility and inability to identify allelic variation in heterozygotes. RAPDs were used for genetic studies in date palm (*Phoenix dactylifera*) [9] and chrysanthemum (*Dendranthema grandiflora*) [10].

Simple sequence repeats (SSRs) are repeat based and co-dominant markers. Di, tri and tetra nucleotide repeats are extensively distributed all over the plant genomes. Microsatellite is more powerful marker system due to high level of allelic variation. These are widely used due to DNA usage, low cost for manual assays and high throughput genotyping. SSRs have been widely used in the development of genomic linkage maps, QTLs mapping and many other genetic analysis of germplasm since 1990s. Many plant breeders considered that SSRs are more successful marker system for genetic diversity, identification of hybrids, classification and DNA fingerprinting of germplasm. SSRs have been extensively used for genetic analysis of many horticultural crops including pear (*Pyrus communis*) [11], grapes (*Vitis vinifera*) [12], cucumber (*Cucumis sativus*) [13] and sugar beet (*Beta vulgaris*) [14].

Inter simple sequence repeats (ISSRs) are also dominant markers [1]. These markers do not distinguish clear difference between homozygotes and heterozygotes genomes. Mostly, these markers produce multiple DNA alleles and larger number of loci across the genome of any species without prior information of DNA sequences of the target regions. These have been commonly used for assessment of genetic variability, taxonomic studies, phylogenetic analysis and DNA fingerprinting of horticultural crops i.e., banana (*Musa paradisi*) [15], bitter gourd (*Momordica charantia*) [16], rose (*Rosa indica*) [17], tomato (*Solanum lycopersicum*) [18] and Indian Jujube (*Zizyphus mauritiana*) [3] etc.

5 Functional Markers

Directed amplification of minisatellite DNA (DAMDS) markers have been used in eukaryotic genomes to detect polymorphism [19]. High level of polymorphism is a common feature of tandemly arranged repetitive sequences which is mostly due to variation in number of tandem repeats and internal structure of individual repeats [20]. The term mini-satellite was firstly used by Jeffreys et al. [20]. DAMDs were developed and used to show the amplification of mini-satellite regions of genome first time [19]. These markers have been used throughout the genome of many species e.g., tomato [21].

Sequence characterized amplified regions (SCARS) are co-dominant markers. These markers are very informative for physical mapping, describing locus specificity, comparative mapping and homology studies among related plant species [22]. SCARs have been involved in cloning of amplified products of arbitrary markers and then sequencing the two ends of the cloned products. The sequence is later used to develop specific primers nearly of 15–30 bp. These primers could further used to amplify the single major fragment similar to that of cloned allele. SCARs have also been used to enhance decision making

as well as timely disease management such as *Pseudofabraea citricarpa* on citrus (*citrus sinensis*) [23]. Recently, SCARs were used for evaluation of genetic variation in chrysanthemum flower shape [24].

Cleaved amplified polymorphic sequences (CAPS) are co-dominant and of locus specificity nature. These markers are implemented through digesting locus-specific PCR products with one or more restriction enzymes, followed by separation of the digested DNA on horizontal or vertical gels. These have been used to differentiate between plants that are homozygous or heterozygous for alleles [25]. Therefore, these markers have been helpful for genotyping, map based cloning and identification of germplasm. These markers are very simple, low cost, using universal PCR technologies, restriction digestion and agarose gel analysis. Nineteen CAPS markers were used to identify the parentage in citrus cultivars [26]. These markers are very useful for assessment of mutations in segregating populations and positional based cloning of new genes in plants.

SSRs based markers provide a high level of polymorphism, however these are labor-intensive. RAPDs are low costs but show a low level of polymorphism. To control the flaws of these two marker systems, RAMPs were developed [27]. RAPMs were used for evaluation of genetic variation in peach germplasm [28].

Sequence-related amplified polymorphisms (SRAPS) are co-dominant in nature. SRAPs provide easiness, reliability and superficial sequencing of particular alleles [29]. Changes occurring in allele size due to insertions and deletions of nucleotides led to co-dominant markers, while alterations in nucleotides led to dominant markers. SRAPs used for construction of genome mapping, tagging and genetic diversity analysis in okra [30].

Target region amplification polymorphisms (TRAPS) are quick and effective markers that use bioinformatics tools and express sequence tag (EST) database information to produce polymorphic markers, around targeted candidate gene sequences [31]. TRAPs have been extensively used to produce markers for specified gene sequences [32]. These are also very helpful for germplasm classification and producing markers linked with desired traits in various crops for marker-assisted breeding. These markers have been successfully used for DNA fingerprinting of lettuce (*Lactuca sativa*) germplasm [32].

Single strand conformation polymorphisms SSCPs are simple and efficient markers which depend on the mobility change of single stranded DNA on non-denaturing polyacrylamide gel [33]. These markers have been used for genetic analysis and particularly played role in analysis of molecular heterogeneity of grapevine virus A [34].

The advent of organelle microsatellites have been widely used for the study of population genetic structure and phylogenetic relationships in plants on the basis of plant organelle genomes. Plant organelle genomes show different arrangements of genetic variation as compared to nuclear alleles [35]. So, these advanced markers play significant role for a complete understanding of plant population variation and evolution. Organelle microsatellites have been further classified into chloroplast microsatellites and mitochondrial microsatellites.

Chloroplast microsatellites are used to identify genetic gap between taxa. The slight morphological diversity can be attained through chloroplast microsatellites which cannot be revealed through nuclear DNA markers for interbreeding and genetic exchange. These markers are successfully used as a high-determination tool for evaluation of cytoplasmic variation, revealing of hybridization and introgression, analysis of genetic diversity and phylogenetic studies in a wide range of plant species [36]. Many chloroplast microsatellites have been developed for genetic analysis of various plant species such as in families *Poaceae* and *Solanaceae* [37,38].

Mitochondrial microsatellites are not used for phylogenetic studies in plants because of high rate of sequence reorganization [39]. Though, mitochondrial haplotype diversity linked to sequence rearrangement has been very helpful in population variation of pine and fir taxa and these are also used for trait-based discrimination of population [40].

6 Specific Sequence Based Technologies

The arrangements of nucleotide bases i.e., adenine, guanine, cytosine and thymine along the DNA molecule is known as DNA sequencing. Specific sequence based markers mostly depend on documentation of a specific DNA sequence in a gene pool of unknown DNA [41]. The development and advent of these specific markers proved that non-PCR based or hybridization markers are unreliable as well as less polymorphic. Introduction of different sequencing technologies and their application methods i.e., Sanger method of sequencing, next-generation sequencing, pyrosequencing and genotyping by sequencing provide a great revolution to breeders for development of SNPs which detect high polymorphism. Sequence based markers have been incorporated to enhance detection of genetic gap and uniqueness in studied germplasm. Therefore, these advanced molecular marker have effectively been applied for genomic studies of individuals, genera or species. Moreover, few imperative sequencing approaches are also listed below.

Sanger's method of sequencing is known as first generation of DNA sequencing techniques. Firstly, Sanger et al. [42] developed and used this sequencing technique. The main principle of this technique is that single stranded DNA fragments which express length variation of a single nucleotide detached from each other. PAGE can be used for separation of single nucleotide. In this method of sequencing modified bases are mostly used. Therefore, this method is also famous as Sangers's dideoxy sequencing method. In this technique, oligonucleotides were sequenced through polymerization by enzymes for genome sequencing [43]. Huge variation evaluated using such sequencing technologies. Sequencing approaches are found be very reproducible and need a small quantity of DNA. However, sequence technology is expensive, time consuming, low genomic coverage and low polymorphism distinguished below the species level.

Pyrosequencing is non-electrophoretic, sequencing - by - synthesis technique used for detection of phosphate group during DNA synthesis. Hence, it is a synthesis principle-based sequencing technique. This technique is found to be very effective for characterization of nucleotides. It performance in determination of complex DNA structure i.e., cDNA analysis, mutation exposure, re-sequencing of diseases genes, viral typing, bacterial typing and SNPs [44]. Pyrosequencing has been further categorized into two major techniques i.e., solid phase and liquid phase sequencing. Pyrosequencing has greater potential of accuracy, easy handling and can be simply programed. Moreover, this method of sequencing restricts the requirements of labeled markers, labeled bases and gel electrophoresis [45]. This technique has been more satisfactory for confirmatory as de novo sequencing [46]. De novo sequencing was used for genetic analysis of date palm germplasm [47]. In future, it has been expected that pyrosequencing will decrease sequencing time, reduce sample quantity and bring more improvements in automation.

Next-generation sequencing (NGS) is known as high-throughput sequencing technique. The advent of these high throughput sequencing techniques allow researchers to sequence DNA or RNA more quickly low cost. It is involved in covering of whole genome sequencing more accurately to examine the huge number of SNPs used for evaluation of genetic diversity and genome wide association studies [48]. So, these sequencing techniques bring a great revolution in molecular genetics. Next-generation sequencing is involved in full genome sequencing or more targeted discovery of mutations or polymorphism. It is also helpful for large scale analysis of DNA methylation. Moreover, this sequencing technique has greater potential for production of billions of DNA bases per run. Different organizations have successfully developed these techniques and provide their services commercially. Similar procedure in all NGS systems for synthesis of template DNA. NGS was used to establish a relationship on the basis of genotypic and phenotypic traits in apple [49]. Phenotype-genotype association was utilized in genome-assisted breeding that enhanced the breeding of excellent and high yielding cultivars. This sequencing technique is done in constant channel and one or more nucleotides are incorporated, resulting in the release of a signal that is successfully identified by a sequencer [50].

Genotyping by sequencing (GBS) is very effective and helpful techniques for genome sequencing of horticultural crops. Advances in next-generation sequencing reduced sequencing cost and also ensure the positive use of GBS for diversity assessment in large genome species [51]. GBS has been categorized into two types i.e., restriction enzyme and multiplex enrichment PCR. Restriction enzyme is used for new markers identification and their application in MAS [52]. GBS was successfully used for high-resolution genotype and trait association of those species having complicated genome. Major advantages of GBS include; low sequencing cost, easy sample handling, satisfactory results in breeding of diverse crops, less PCR and purification set required [53]. Extensive was recorded between diploid roses using GBS with development of high density genomic map [54]. Application of GBS and SNPs are considered as useful resources for genetic improvements of economical traits of almond [55]. Following SNPs markers have been developed through above mentioned sequencing techniques.

Single nucleotide polymorphism (SNPs) are single nucleotide based variation in two DNA sequences or individuals. These are co-dominant markers. Therefore, these are able to differentiate between homozygous and heterozygous fragments. These are the most abundant and effective markers for plant genetic studies. Recently, SNPs emerged as the new and latest generation molecular markers for plant breeding programs [56]. These markers have been used to assess the large number of loci. SNPs are the utmost significant markers due to fast and effective DNA fingerprinting of large numbers of genotypes [57].

Diversity array technology DArT provides a greater opportunity for evaluation of genotyping of polymorphic loci which are scattered over the genome. Moreover, it is one of the highly reproducible microarray hybridization tools. Prior sequence information is not required for evaluation of gene interest [58]. The most advantage of DArT is that it is very economical as well as high throughput technique. Very small DNA (50 - 100 ng) is required and sufficient for genotyping purposes. Different sequencing technologies are considered as an efficient platform for development of molecular markers. These polymorphic markers can be successfully utilized for genotyping of huge set of genotypes. Different molecular markers have been used for numerous genetic analysis of horticultural crops (Tab. 1).

7 Use of Targeted Genome Editing Tools

Recent advances in genome editing brought new prospects for forward and reverse genetics in genomic studies. Genome editing technologies based on three basic components i.e., CRISPR/Cas, TALENs and ZFNs are successfully applied in horticultural crops [59]. CRISPR technique made a great revolution in plant breeding for different horticultural crops. CRISPR technique is successfully applied for genome editing of plants, animals, bacteria and fungi. Moreover, genome selection and genome editing might be collectively utilized for development of improved genotypes. Genome editing reduces backcrossing time performed between elite genotypes and exotic germplasm. Moreover, exotic germplasm used as encyclopedia for development of resistant genotypes against different stresses. Genome selection is used after the selection of recombinant alleles. Genome editing is performed in economically important horticultural crops. CRISPR/Cas9 technique is successfully used for accurate genome editing in grapes [60]. CRISPR/Cas9 directly targeted DIPM-1, DIPM-2, and DIPM-4 genes in apple plants to enhance tolerance against fire blight disease [61]. CRISPR/Cas 9 technique was successfully used to induce targeted mutagenesis in 1st generation of pear and apple transgenic lines [62]. TALEN reagents and CRISPR/Cas9 were utilized for evaluation of diploid as well as tetraploid potato clones to improve tolerance against cold-induced sweetening, improved starch quality and self-incompatibility [63].

Table 1: Commonly used molecular markers for genetic analysis of horticultural crops

Marker type	Fruit name	Function
RFLPs	Citrus, date palm, mango and Indian jujube	Genome mapping and disease analysis
AFLPs	Sweet potato, roses, mango, tomato, mandarin, olive, apple and grapes	Genotyping of species or their individuals, genome mapping and transcript profiling
RAPDs and ISSRs	Guava, marigold, broccoli, citrus, potato, mango, chrysanthemum, apple and pistachio	Genetic diversity, phylogeny, genomics and evolutionary relationships
SSRs	Banana, citrus, cucumber, tomato, mango, date palm, grapes, peach, plum and pear	Genetic variability, development of genomic linkage maps and QTLs mapping
SNPs	Tomato, eggplant and radish	Genome wide association studies
RAMPs	Peach	Population structure and genetic variation
SCARs	Chrysanthemum, eggplant and banana	Comparative mapping
SRAPs	Citrus, grape, eggplant and chrysanthemum	Genome mapping and genome tagging
CAPS	Citrus	To identify parentage
SCoTs	Roses, citrus and Indian jujube	Identification of germplasm
SSCP	Grapevines	Heterogeneity of grapevine virus A
DArT	Citrus and tomato	Gene map construction for fruit quality
MAS	Potato, cumber, grapes and citrus	Detect disease resistance genes
Genome sequencing	Tomato and date palm	Genome wide association, DNA methylation and targeted mutation
QTLs	Tomato, citrus and grapes	Detection of gummosis and salt tolerance
CRISPR/Cas9	Citrus, date palm, apple and potato	Tolerance against fire blight disease
Functional genomics	Date palm, grapes and citrus	Distinguished genes coding for salt tolerance in genome regions
Genetic transformation	Banana	Musa DHN1 gene expression enhanced salt tolerance by increasing proline
Transgenic plants	Grape, apple, plum, banana, melon, petunia, carnation, potato and peas	Heritable resistant against powdery mildew

8 Mapping and Tagging of Genes

Gene mapping is used to detect the gene locus, distances among genes and linkage among numerous markers and desired traits. Gene tagging is detection of DNA sequences in genome that can perform as a tag for desired genes. Molecular markers were used for genome mapping of roses [54]. First comparative genomic map was constructed between *Poncirus* and *Citrus* species [64]. RFLPs were successfully used for genome mapping of rose and related species [7]. SSRs were used to investigate the genome mapping in citrus [65]. Six types of DNA based markers were used to construct a new genomic map of citrus derived from mandarin *(Citrus reticulata*) and tangelo (*Citrus reticulata*) [66]. Linkage mapping of initial blooming time and flowering duration in chrysanthemum proved that SRAPs were significantly

linked with morphology [67]. Therefore, SRAPs are very helpful in developing breeding programs of chrysanthemum in future. Co-dominant markers were used to achieve comparative genome mapping of pummelo, sweet orange and clementine [68]. Transcriptome sequencing was used for improvement in functional genes related to salt stress [69]. The genomes of wild and cultivated tomato germplasm were analyzed to detect the genomic regions that faced variation during domestication [70].

9 Evaluation of Genetic Diversity

Information regarding the extent of genetic variation within or among the genera, species or individuals plays an essential role in planning future breeding programs. It is a significant pre-requisite to get progenies with desired traits especially higher yields. RFLPs have been used for assessment of genetic diversity in Indian jujube [71]. Date palm cultivar "Medjool" is a landrace resulting from a mixture of genotypes that grown mostly by natural selection and confirmed through AFLPs [72]. Comparative evaluation of guava (*Psidium guajava*) germplasm was conducted through RAPDs [73]. High level of genetic variability was detected among three local and five imported mango cultivars [74]. Estimation of genetic diversity in 70 mango (*Mangifera indica*) genotypes was evaluated using 33 ISSRs [75]. High level of genetic diversity among ten genotypes of *Zizyphus nummularia* was identified using morphological and ISSRs [76]. Genetic differences were detected among 41 tomato genotypes using eleven ISSRs [18]. ISSRs were successfully used for evaluation of genetic variation among rose genotypes [17]. Genetic structure of eight tomato cultivars was developed through ISSRs and RAPDs [77]. Evaluation of genetic variation in 56 pear genotypes was assessed through 12 SSRs [11]. Genetic association was evaluated among 60 genotypes of date palm by using 17 SSRs [78]. EST-SSRs have been used for analysis of genetic diversity in mango genotypes [79]. A set of 23 SSRs was used for analysis of genetic variability among 104 cucumber genotypes [80]. Genetic diversity was evaluated in order to get new genetic combinations among 13 genotypes of sugar beet through 14 SSRs [14]. Genetic differences and population structure were assessed among varieties of *Luffa* species through 103 SSRs [81]. Genetic diversity was examined among 36 genotypes of pineapple (*Ananas comosus*) using 20 SSRs and 13 ISSRs [82]. SRAPs were applied for determination of genetic diversity and population structure among wild, ornamental and cultivated genotypes of pomegranate (*Punica granatum*) [83]. Genetic diversity was evaluated in 37 Indian jujube genotypes through SCoTs and ISSRs [84].

10 QTLs and Association Mapping

Traits of horticultural crops are found to be polygenetic in nature. These traits are controlled through different genes on same or different regions of chromosomes. QTL mapping is an efficient method in which different molecular markers are used to detect the genes that affect the desired traits. Association mapping is an efficient technique to detect QTLs in horticultural crops. Traits have been categorized into two groups as quantitative and qualitative traits. Continuous variation was observed in quantitative traits, while discontinuous variation in qualitative traits. So, molecular markers are more efficient and helpful tools for QTLs evaluation as well as MAS. QTLs mapping mainly involves during diverse parent selection having genetic variation that affect the desired traits. QTLs mapping is constructed on phenotyping and genotyping data of mapping population. Moreover, polymorphic markers are needed for QTLs mapping. Then, genomic map is constructed and various statistical databases are used to detect genetic markers associated with desired trait [85]. A wide series of SSRs was used for QTLs mapping of F_1 tomato hybrids [86]. A set of 87 SSRs were used for QTLs mapping of 60 mango cultivars collected from different regions [87]. DArTseq markers were used to construct the genetic maps of *Citrus sunki* and *Poncirus trifoliata* and compared with *Citrus sinensis* genomic map [58]. Tomato linkage map was constructed using 172 SSRs, 3 SNPs and 2 morphological markers and proved that linkage map is more helpful to enhance fruit quality and reduce chances of linkage drag [88]. QTLs and eQTLs mapping related to 'citrandarins' detected that gummosis resistance is due to

both parents that provides favorable transmission to their hybrids [89]. Genome wide association was constructed regarding fruit quality traits of citrus using GBS and SNPs. However, four QTLs for fruit weight and one for fruit color and firmness were identified. These QTLs are found to be novel and can be utilized for cross breeding of citrus [90].

11 Evolution and Phylogeny

Earlier theory of evolution was developed on the basis of morphology. With the improvement in molecular markers, conformation of genetic structure of natural population became clear and gave necessary information regarding the type of genetic variation linked with species divergence. RFLPs were used to detect genetic relationship among 10 poly-embryonic and 10 mono-embryonic mango cultivars [91]. Genetic relationship was detected among 148 genotypes of pineapple through AFLPs [8]. Phylogenetic relationship was determined among 19 genotypes of peppers (*Capsicum annuum*) by using 56 AFLPs [92]. SSRs were used for evaluation of phylogenetic relationship among banana genotypes [93]. ISSRs and RAPDs have been commonly applied for assessment of genetic diversity, phylogeny, genomics and evolutionary relationships [94]. Genetic relationship was identified among commercial cultivars and wild species of grapes using SRAPs [95]. Phylogenetic association was recorded among 16 grapes genotypes using 9 SSRs [96]. SNPs were used to examine the population structure and evolutionary pattern in eggplant (*Solanum melongena*) genotypes. SSRs and SNPs were used in combination to find out genetic relationship among eggplant genotypes [97]. Expressed sequence tags (EST) were applied for assessment of evolutionary relationship among date palm genotypes [98]. Phylogenetic relationship was examined among 19 tomato cultivars by using 20 RAPDs [99].

12 Hybrids Identification and Genetic Purity Tests

Genetic purity is very important component during hybrid seed production for commercial purposes. Genetic purity test through morphological characterization of hybrid progeny is very difficult because it takes few months in field trials during evaluation (15, 100). Therefore, molecular markes should be used for pecise identification of hybrids and also test purity in hybrid seeds. Molecular markers have been used for identification of hybrids and purity testing of vegetable crops. Cucumber hybrids were identified through RAPDs [101]. ISSRs have been successfully used for identification of banana genotypes or clones [15]. Interspecific hybrids of eggplant were identified using ISSRs [102]. Genetic purity was tested in three eggplant hybrids by using six SSRs [103]. ISSRs were used for seed purity testing of bitter gourd hybrids [16]. EST-SSRs were very efficient markers to test genetic purity of F_1 seeds of melon "Green Angle". Twenty RAPDs were used for identification of tomato hybrids and their performance [104]. The successful hybridity was evaluated in chilli (*Capsicum frutescens*) hybrids through 27 RAPDs and 20 ISSRs [100]. Seven SSRs were applied for the identification of hybrids among 42 genotypes of grapes [12].

13 Gender Discrimination and Diseases Diagnosis

Many woody dioecious plants cannot be identified as female (productive) and male (unproductive) at seedling stage. Therefore, selection of productive plants for commercial production is a big problem. Molecular markers can resolve this issue and identify male and female plants at any stage [105]. Morphological, physiological and biochemical markers were used to determine sex expression in papaya [106]. Female and male plants of papaya were identified using 37 SSRs [107]. RAPDs have been used for sex determination in pistachio (*Pistacia vera*) plants [108]. Sex expression has also been identified in date palm plants through molecular markers [109]. CRISPR/Cas9 based techniques is more helpful in sex determination and successfully used in date palm [110]. Molecular markers can also be used for sex determination in cucumber [111].

Diagnosis of plant diseases in horticultural crops is very essential for their proper management. Molecular markers can be used to identify pathogens in a quick way. These markers enable the plant

breeders to develop disease resistant plants. AFLP markers were used for identification of disease resistant and disease susceptible varieties in cucumber [112]. Fungi isolates have been detected on grapes through RFLPs [113]. Molecular markers have been used for identification of mango malformation. Correct mango malformation detection through molecular markers has also studied [114]. Recently, NGS provides quick genomic region identification linked to preferred phenotypes in many species [115]. Thirty SSRs were applied for identification of mango hybrids and landraces as well as their susceptibility to mango malformation. Amrapali, hybrid of Neelam × Dashehari, had more resistance against malformation in the studied germplasm [116]. Morphological markers and internal transcribed spacer method found that stem disease on wild citrus was anthracnose produced by *C. gloeosporioides* [117]. Timely and right diagnosis of diseases is very significant to control the disease spread particularly to a new area. STS and SSRs were used to develop genome mapping of onion. Monogenic dominant gene ApR_1 is involved to control purple blotch disease in onion genotype 'Arka Kalyan' [118]. A set of 28 ISSRs and 16 AFLPs were applied for identification of molecular markers linked with anthracnose resistance in chillies [119]. Several chillies genotypes i.e., Perennial, C00226, JCA-288, Sel 6, S-343, Japani Longi, Sel 15 and Punjab Tej were found to be resistant against nematodes as well as leaf curl virus in India. One genotype 'Anugraha' has resistance against bacterial wilt and 'CM334' showed better resistance against phytophthora root rot [120]. Thus, these genotypes can be further utilized in breeding programs.

14 Molecular Basis in Salt Tolerance

Reduction in plant vegetative and reproductive traits may possibly be resulting in poor crop yield due to salts toxicity. Different molecular techniques can be used to evaluate tolerance mechanism of crops and identification of salt resistant genes. However, genetic transformation is efficient way for development of salt tolerant genotypes. Polygenic nature is a major limitation for development of salt tolerance cultivars [121]. However, one or more traits can be improved using different physiological and molecular markers [122]. Moreover, salt tolerance mechanism in several fruit crops is still unclear. The advent of genome linkage maps provides a great revolution to resolve such issue in woody perennial crops [123]. Limited research work is available for evaluation of salt tolerance mechanism and development of salt resistant germplasm of horticultural crops. The screening of genotypic performance under salt stress in field conditions is laborious, time consuming and inaccurate. However, molecular markers can be used for evaluation of salt resistant or sensitive genotypes in short working duration. The development of salt tolerant genotypes through QTLs and molecular markers is quite slow in horticultural crops. A lot of limitations occur through traditional breeding and do not provide accurate outcomes. Gene identification through molecular markers and their cloning against numerous biotic and abiotic stresses is progressively increased. Approximately, 98 QTLs were explored to evaluate salt tolerance in citrus hybrid [Cleopatra mandarin (salt tolerant) × Trifoliate orange (salt sensitive)] [123]. RAPDs (OPC-02) were successfully used and distinguished the salt tolerant date palm genotype Bugal White [124]. Application of molecular markers in salt tolerance mechanism of horticultural crops was presented (Tab. 2).

15 Marker-Assisted Selection

MAS provides significant approaches to facilitate the mechanism of breeding in horticultural crops. MAS is effective tool for examination of thousands genome regions for improvement of economically important traits under water and salt stress conditions. RFLPs, APLPs, RAPDs, SSRs and ISSRs are efficiently used for different genetic analysis of plants. These markers are not developed from the genes because gene cloning is more complicated in polyploid crops having large size of genome. On the other hand, functional markers have been developed form polymorphisms within transcribed regions of functional genes. These markers are significantly associated with function of genes. Functional markers are directly involved in precise selection of targeted genes. Genetic variation and novel alleles exist in wild plants. Thus, wild plants are considered as potential source of gene(s) for producing resistance

Table 2: Application of markers technology for genetic studies of horticultural crops

Molecular marker	Function	Reference
AFLPs, RAPDs and ISSRs	These can be used for conservation of rare species	[5]
Functional markers	RAPDs were used to distinguish genes responsible for salt tolerance in date palm	[124]
QTLs	QTLs mapping revealed 70 potential QTLs in 16 regions of citrus genome and 6 of them were involved in growth as well as dry weight production	[28]
Association mapping	Association mapping exhibits genetic variability across a natural population	[123]
Genome based sequencing	Whole genome sequencing is successful for evaluation of genes number and structure and also their association on the chromosome in a specific genome	[129]
Genome tagging	Tagging through transposable elements or T-DNA constructs may have greater potential to detect the essential function of a particular gene by uncovering a particular phenotype	[130]
Gene expression	Nearly, 21 HAK/KUP/KT genes were detected in pear genome involved in K^+ deficiency.	[131]
Genomic and proteomics	Proteomic approaches encode the whole set of proteins which are found in biological sample	[132]
	35 protein spots were detected in salinized date palm plants out of 55 protein spots	[133]
Genetic transformation	Over expression of Musa DHN1 gene enhanced salt tolerance by increasing accumulation of proline in transgenic banana leaves	[134]
Transcriptional analysis	Transcriptome analysis were used for comparative evaluation of dwarf as well as standard pear rootstocks	[135]
Genome editing	It reduces backcrossing time between elite genotypes and exotic germplasm.	[60]

against biotic and abiotic stresses. Application of functional markers and genetic transformation are valuable resources for desired genes transfer from wild to cultivated species. Crossing, selection and molecular breeding are used to facilitate the transfer of abiotic stress resistance from wild to cultivated species. Molecular markers are successfully used to enhance backcrossing of genes from wild species to inbred lines or elite cultivars. First genetic map of potato was constructed on sexual recombination frequencies in 1980's. MAS have been used by potato breeders to improve economically important traits and also helpful to detect disease resistance genes [125]. Functional marker "NL25" was successfully detected candidate gene having resistance characteristics against potato wart [126]. MAS is used to identify potential genes in water melon (*Citrullus lanatus*) against *Fusarium oxysporumi* [127].

16 Proteomic and Transcriptomic Approaches

Molecular research for different genetic analysis is an urgent need of time to understand the plants. Proteomics are essential tools for evaluation of particular species or cultivars and their specific genes. Proteomic analysis is found to be efficient and useful for identification of metabolic mechanism involved

in improvement of fruit quality. Protein are involved in different biotic and abiotic plant stresses and also involved in their defense mechanism. Under salt stress, 35 protein spots were identified in date palm plants [133]. Proteomic approaches were successfully used for early evaluation of phyto-infestation caused by red palm weevil in date palm plants [136]. So, proteomic approaches can be used for selection of resistant cultivars against biotic and abiotic stresses. Proteomic approaches can be used to understand complex mechanism of fruit ripening and mesocarp development [137]. These approaches are directly involved in plant response under stress that can be detected. Huge set of proteins can be encoded through proteomic approaches which involved in regulation of plant stress.

Transcriptomic studies can be used for evaluation of novel genes responsible for biotic and abiotic stresses [138]. 21 HAK/KUP/KT genes were evaluated which are responsible for K^+ deficient and other abiotic responses in pear genome [131]. Recently, transcriptome analysis were used for comparative evaluation of dwarf as well as standard pear rootstocks [135]. List of software's used for analysis of genomic data of horticultural crops were listed (Tab. 3).

Table 3: List of softwares and their operating systems commonly used for analysis of genomic data of horticultural crops

Software name	Operating system	Description	Reference
NTSYSpc Version 2.0	Windows	Performed cluster analysis for evaluation of genetic similarity/ dissimilarity	[1]
NTSYSpc Version 2.2	Windows	Performed cluster analysis for evaluation of genetic similarity/ dissimilarity	[13]
STRUCTURE v2.3.4	Windows and UNIX	Evaluation of allelic admixture among individuals or their species	[81]
POPGENE 1.32	Windows	Effective number of alleles were calculated	[13]
MEGA v5.2.2	Windows and MacOS	Phylogenetic relationship can be determined	[139]
STATISTIX 8.1	Windows	It can be used for evaluation of genetic variation from physico-chemical data	[3]
XLSTAT, 2019	Windows	Genetic variation and correlation matrix were calculated	[3]
R PACKAGES	Windows, NT, Ubuntu and UNIX	Trait association analyses can be constructed	[140]
IMAGEJ	Windows	Fruit and seed analysis can be performed	[141]
MINITAB, 2019	Windows	Phylogenetic relationship can be determined	[3]
SNPEFF 3.4	Windows	Genome mapping of individuals and species	[142]
JBROWSE 1.10.12	Windows	It can be used to visualize the detected structural variants	[142]
CLUSTALX	Windos, MacOS, UNIX	Offers multiple alignment of DNA sequences	[139]
LAMINA	Windows	A tool for rapid quantification of leaf size and shape parameters	[143]

17 Implications for Genome Conservation and Management

Information regarding existing genetic variation within germplasm and natural population is most important component for planning of in situ and *ex situ* conservation approaches. Molecular markers are very efficient tools to explore genetic variation among wild species for future breeding programs [139]. All molecular markers are very helpful in identifying duplicates among core collection and in this way efficacy of management is enhanced. When core collection is identified then molecular markers are used to identify genetic variation, which reduces chances of genetic erosion [35]. AFLPs, RAPDs, and ISSRs do not require any sequence data and their genomic coverage is also high. These can be used for conservation of rare species. Genomic coverage of SSRs is moderate but sequence data is also not required to detect variation among the individuals.

18 Conclusion

Molecular markers can be used for evaluation of germplasm to enhance crop production. All the wild species of horticultural crops need to be characterized through molecular markers for evaluation of DNA fingerprinting and identification of disease resistance genes which can be utilized in breeding programs. Sex expression can be identified through molecular markers in diocious plants at early stages of growth. Wild germplasm within country has greater potential regarding tolerance against biotic and abiotic stresses with excellent nutritional components. Therefore, wild germplasm should be utilized for breeding purposes to incorporate resistant genes in crop plants. DNA based markers especially sequence based markers can be used in plant breeding for crop improvement programs. These markers can identify genes, which are liable for expression of desired traits. The selection of suitable and appropriate markers should be based on various factors comprising the availability of technology, costs for marker development, species transferability and ease of certification. Conclusively, DNA fingerprinting, bar coding, genome sequencing and re-sequencing studied are excellent revolution for improvement of economically important traits in horticultural crops. Innovation in markers technology is accurate, cost effective and highly reproducible to evaluate the desired genotypes with excellent traits.

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