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Review Article

Applications of Molecular Markers to Assess Genetic Diversity in Vegetable and Ornamental Crops – A Review

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ABSTRACT

Assessment of genetic diversity has attained much consideration during the last two decades for efficient germplasm management and its utilization in breeding programs. Molecular markers system is very helpful in correct identification of plants, successful management of plant resources, and to achieve various aspects of breeding programs in vegetables and ornamental crops. Applications of molecular markers for appraisal of DNA variations in plants provide significant approach in field of molecular genetics. Morphological markers are not appropriate for evaluation of genetic diversity due to less differentiating traits among species, genera or their individuals. These are also highly affected by climatic factors. So, molecular markers system is very effective method for detailed DNA finger printing of crop plants. However, successful use of molecular markers in crop breeding programs relies on strong coordination among plant breeders, biotechnologists and trained manpower as well as proper financial support. The current review explains the basic descriptions of different molecular markers and their applications for genetic improvement programs in some vegetables and ornamental plants.

Keywords: *DNA finger printing, genetic diversity, genetic improvement programs, germplasm characterization, morphological markers.*

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Abbreviations: **AFLPs**, amplified fragment length polymorphisms; **DAMDs**, directed amplification of minisatellite DNA regions; **ISSRs**, inter simple sequence repeats; **PCR**, Polymerase chain reaction; **QTLs**, Quantitative trait locus; **RFLPs**, Restriction fragment length polymorphisms; **RAPDs**, Random amplified polymorphic DNAs**;** **RAMPs**, Randomly amplified microsatellite polymorphisms; **SSRs**, Simple sequence repeats; **SSCPs**, Single strand conformation polymorphisms; **SNPs**, Single nucleotide polymorphisms; **SRAPs**, Sequence-related amplified polymorphisms; **SCARs**, Sequence characterized amplified regions; **SCOTs**, Start codon targeted.

INTRODUCTION

Since ancient times, morphological markers were used to measure the genetic variations as well as desired traits (Alcaraz and Hormaza, 2007; Anjum et al., 2018). The use of morphological markers is being eliminated because these are affected by environmental condition and developmental stages, cause troubles during the identification of homozygous and heterozygous individuals. Molecular marker is a specific fragment of DNA that is representative of the variations at genome level. The advent of molecular techniques over the last few years has provided easy and accurate identification of plant species and genera. Molecular markers have been used for characterization of germplasm, evaluation of genetic diversity, identification of cultivars, clones or hybrids, assessment of genetic relationship, phylogenetic analysis, evolutionary relationship, taxonomy, gene mapping and genome tagging (Zhang et al., 2010; Yang et al., 2015). During last three decades, DNA based markers and recombinant DNA technology have been extensively used for construction of genomic, cytogenetic and physical maps of crop plants (Korzun, 2002). The ideal properties of molecular markers include reproducibility, dominant or co-dominant inheritance, high level of polymorphism, low cost, easy access and transferability among laboratories (Mondini et al., 2009). Careful selection of molecular markers is important because no molecular marker fulfils all these characteristics. Horticultural crops have also received attention during last few years in the field of molecular markers but only few attempts have been made in vegetable and ornamental crops (Kour et al., 2011). Different types of molecular markers have been used for appraisal of genetic diversity in vegetables and ornamentals, as presented in Table 1 and 2.

CLASSIFICATION OF MOLECULAR MARKERS

Non-PCR Based Markers

The first genetic marker developed and used for detection of DNA variations and construction of genomic maps in humans was restriction fragment length polymorphism (RFLP) (Botstein et al., 1980). Later, many molecular markers were developed for plant genetic analysis (Zietkiewicz et al., 1994). RFLPs are used for evaluation of genetic diversity and to examine the

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relationship of closely linked taxa (Dijkhuizen et al., 1996).

PCR Based Markers

PCR based markers extensively used in genetic studies include random amplified polymorphic DNA (RAPD) (Bardakci, 2001), amplified fragment length polymorphism (AFLP) (Aktas et al., 2009), microsatellites (SSRs) (Ahmad et al., 2012) as well as inter simple sequence repeats (ISSRs) (Bornet et al., 2002).

Sequence based markers

The variations of single nucleotide (A, T, G, C) in arrangement of plant genome are considered as sequence-based markers and are called single nucleotide polymorphism (SNPs) (Clarke et al., 2009). Mostly, these markers are established from either genomic DNA libraries (RFLPs, SSRs) or from random amplification of genomic DNA (RAPDs).

APPLICATIONS OF MOLECULAR MARKERS

Vegetables

The effectiveness of a breeding program in a vegetable mainly depends on the availability of polymorphism of that crop and breeding success also depends on genetic diversity. Recently, genetic diversity faced the problems/issues of genetic losses due to commercial cultivation of high yielding uniform cultivars, elimination of natural habitations of fauna and flora due to urbanization and industrial development. Therefore, conservation and effective use of genetic resources is a basic need for crop improvement programs. Molecular markers accelerate the breeding processes by marker assisted selection, phylogenetic studies and DNA fingerprinting of germplasm. The application of molecular or genetic markers is based on naturally occurring DNA polymorphism. The achievements made in characterization of vegetables through molecular approaches are presented in Table 1.

Potato

DNA fingerprinting, identification and taxonomy of potato cultivars is very complicated due to hybrid origins and evolutionary aspects of current hybridization (Raker and Spooner, 2002). Therefore, different molecular markers i.e. AFLPs, RAPDs, SSRs and ISSRs have been effectively used for genetic analysis in plant breeding and germplasm management (McGregor et al., 2000; Ghislain et al., 2004). Bornet et al. (2002) used 77 ISSRs for characterization of 28 potato cultivars collected from different fields. Ghislain et al. (2004) concluded that SSRs provide maximum genetic information, highly reproducible and easy to use for analysis of potato genetic resources. Applications of molecular markers are very successful for germplasm characterization either alone or in combinations. However, Gorji et al. (2011) also used three types of molecular markers (SCOTs, ISSRs and RAPDs) in conjunction to detect polymorphism for genotypes and for varieties of tetraploid potato. In previous studies, four types of molecular markers (AFLPs, RAPDs, SSRs and ISSRs) also gave satisfactory results (McGregor et al., 2000).

Tomato

Tomato is a self-fertilization crop species and its germplasm has been reduced by the breeding of new commercial cultivars outside the native regions. Different molecular markers have been applied to measure the genetic diversity among tomato germplasm (Frary et al., 2005). SSRs have been effectively used to examine the genetic diversity in tomato (Benor et al., 2008). Though, the polymorphism level in cultivated tomatoes shown by SSR is very low (Yang et al., 2005). Some studies have also been conducted on assessment of genetic diversity among wild species or between the cultivated tomatoes (Chen et al., 2007; Benor et al., 2008). Chen et al. (2009) examined the genetic variations in 216 tomato cultivars, hybrids and elite breeding lines using SNPs and SSRs. In the studied genotypes polymorphism was 72.3% and polymorphism in individual populations was 51.06-59.57%. However, genetic variations were narrow in all populations.

Pepper

Genetic diversity in peppers has been analyzed through different molecular markers including AFLPs (Aktas et al., 2009), RAPDs (Adetula, 2006), SSRs (Stagel et al., 2009) and DAMDs (Ince et al., 2009). Rai et al. (2013) used 106 SSRs and 17 RAMPs to investigate the genetic diversity and relationship among 48 genotypes of pepper originating from nine countries. All 38 *Capsicum annuum* genotypes and an interspecific landrace grouped together, while nine non-annuum genotypes grouped alone in the dendrogram. In a previous study, Lanteri et al. (2003) also observed genetic variations in landraces of pepper by using molecular markers. Aktas et al. (2009) determine the taxonomic and genetic relationships among 19 Turkish pepper genotypes using 56 AFLPs. The taxonomic relationship and genetic variation among these genotypes were examined with those of 5 foreign pepper genotypes. Genetic relationship among the pepper genotypes was explained by dendrograms, which proved that local cultivars have maximum genetic diversity. Genetic diversity can be increased by combining preferred traits from local and wild populations of diverse regions into breeding lines.

Eggplant

Evaluation of genetic variability among eggplant germplasm is interest for the conservation of genetic resources for breeding programs and to assess the ability to rapidly verify the breeding material. Thus, it is essential for genetic improvement and elite gene exploitation like tolerance genes against biotic and abiotic stresses. Isshiki et al. (2008) studied eighteen cultivars of eggplant to evaluate the phylogenetic relationships and identification of cultivars using 100 ISSRs. The highest percentage of polymorphism generated was 99.1%. Demir et al. (2010) worked on molecular description of eggplant germplasm collected from different areas using SSRs and RAPDs. Primer OPB07 generated 64% polymorphic bands and rest of the primers generated less than 50% polymorphism. Dendrograms were also constructed to study the genetic relationships of all the genotypes. For breeding, it is necessary to identify the variations among cultivars or lines. However, little frequency of polymorphism occurs among cultivars and intraspecific lines of

Table 1: Achievements made in characterization of vegetable crops through molecular approaches.

Vegetable crop	Markers type	Achievements	References
Potato	AFLPs, RAPDs, SSRs and ISSRs	Genetic diversity	Ghislain et al. (2004)
Tomato	RAPDs, AFLPs, SSRs, ISSR and SNPs	Hybrids identification	Benor et al. (2008)
Pepper	AFLPs, RAPDs, SSRs and RAMPs	Genetic variations	Ince et al. (2009)
Eggplant	RAPDs, SSRs and ISSRs	Genetic relationship	Stagel et al. (2008)
Cucumber	AFLPs, RAPDs, SCARs and SSRs	Genome mapping	Dar et al. (2017)
Peas	SSRs	Genetic diversity	Ahmad et al. (2012)
Broccoli	RAPDs and ISSRs	Classification of germplasm	Lu et al. (2009)
Sweet potato	AFLPs, RAPDs and ISSRs	Genetic diversity	Gichuki et al. (2003)
Ginger	RAPDs and ISSRs	Genetic divergence	Goulao and Oliveira (2001)
Asparagus	RAPDs, SSRs and ISSRs	Genetic variability	Sica et al. (2005)

Table 2: Achievements made in characterization of ornamental crops through molecular approaches.

Ornamental crop	Markers type	Achievements	References
Roses	RFLPs, AFLPs, RAPD, SSR & ISSRs	Phylogenetic relationships	Yan et al. (2005)
Chrysanthemum	RAPDs, ISSRs and AFLPs	Identification of different cultivars	Zalewska et al. (2007)
Carnation	RAPDs, SRAPs and ISSRs	Mapping of plant chromosomes	Fu et al. (2008)
Marigold	RAPDs	DNA fingerprinting	Mor el al. (2008)
Jatropha	RAPDs	Genetic diversity	Subramanyam et al. (2009)

eggplant possibly due to autogamous nature (Stagel et al., 2008).

Cucumber

Cucumber is known as a perfect plant for carrying out genetic research among the species of *Cucurbitaceae* family due to its narrow genome size (367 Mb), maximum gene expression and short life cycle (Lv et al., 2012). Breeding for increasing its yield, improving quality and insects and disease resistant cultivars had become a big goal for breeders all over the world (Yuan et al., 2008). Molecular markers have been used to describe the genetic diversity in cucumber cultivars, even it has narrow genetic base with 3-12% polymorphism (Yang et al., 2015). Several, molecular markers have been applied to assess genetic variation in cucumber germplasm, these include RFLPs (Dijkhuizen et al., 1996), RAPDs (Horejsi and Staub, 1999), SCARs (Horejsi et al., 1999), AFLPs (Bradeen et al., 2001) and SSRs (Miao et al., 2011; Yang et al., 2015). SSRs have been widely used for genome mapping, QTLs association, phylogenetic studies, marker assisted selection, taxonomic studies, evaluation of genetic variability and phylogenetic studies of cucumber germplasm (Yang et al., 2015; Dar et al., 2017). Genetic diversity and population structure are considered as essential studies to enhance the productivity of agricultural crops. The diverse germplasm can be exploited for crop improvement purposes to provide safety to the farmers against biotic and abiotic factors (Govindaraj et al., 2015). Population structure analysis was carried out by using 23 SSRs in 3342 accessions of cucumber from different countries (Lv et al., 2012). Dar et al. (2017) assessed the genomic variations and population structure in 104 genotypes of cucumber through 23 SSRs. The population structure investigation revealed two main populations. Population 1 contained 47 genotypes, 39 genotypes were in population 2, while remaining 18 genotypes were admixtures. Such study offers maximum knowledge for genotype identification, phylogenetic relationship, DNA finger printing, cultivars breeding, and prospect investigation of germplasm in major cucumber growing countries. Pandey et al. (2013) estimated the genetic erosion in cucumber due to cultivation of improved cultivars for high yield and excellent quality and also estimated the genetic diversity among 35 pea genotypes with SSRs. Fifteen SSRs were found to be polymorphic, which divided all the genotypes into two main groups and eight sub-groups. Genetically diverse genotypes can be identified using molecular markers, which can be used to develop parental lines for pea breeding purpose. Recently, Jain et al. (2014) studied genetic diversity among 96 genotypes using 31 SSRs, which can extensively be used in breeding programs. The polymorphic information content varied from 0.01 to 0.56 with SSRs, which indicated that these are highly reproducible, multi-allelic and easy to score loci. Genetic diversity revealed that the gene pool of peas had narrow genetic base ranging 200 to 700 bp. Further, the maximum use of only few parent varieties in breeding programs reduced the genetic diversity (Baranger et al., 2004). Detailed study of genetic diversity was explained by Smykal et al. (2008) by using SSRs and phenotypic based markers on 164 genotypes, while Baranger et al. (2004) studied the genetic diversity among 148 genotypes using 121 polymorphic DNA based markers and protein markers. Jing et al. (2007) evaluated the gene-based sequence diversity of 39 dispersed gene loci in 48 diverse individuals of genus *Pisum.*

Broccoli

Precise documentation and classification of various germplasm resources is essential for cultivar improvement and protection of breeder's rights (Hale et al., 2006). Little variation and relatively high resemblance showed narrow genetic base in broccoli germplasm (Louarn et al., 2007). So, this narrow genetic base is a major cause of difficulty in cultivar identification and to increase variation. The highly polymorphic markers can be helpful in differentiation of germplasm with a narrow genetic base (Hale et al., 2006). Genetic diversity in *B. oleracea* has been analysed with several PCR based molecular markers (Hale et al., 2006; Louarn et al., 2007). Molecular markers are very helpful for expansion of narrow genetic base, heterosis exploitation and parental line selection of broccoli. Molecular markers provide new evidence on the genetic relationships among broccoli germplasm and give significant information for taxonomic and phylogenetic studies in *Brassicas*. Lu et al. (2009) studied 18

genotypes of broccoli using 74 RAPDs and 8 ISSRs markers. The combination of these two markers successfully distinguished the broccoli genotypes into two main sub-groups. Dendrogram indicated that broccoli is most closely related to cauliflower as compared to cabbage and Chinese cabbage.

Sweet Potato

Sweet potato is one of the best vegetables with high nutritional value for human's health (Low et al., 2007). Characterization and distribution of sweet potato germplasm is essential for appropriate exploitation and management. Natural hybridization and selection were involved in hundreds of native sweet potato cultivars in South and Central America, Africa and Asia. These countries are probably considered as main region of diversity of sweet potato (Gichuki et al., 2003). Several studies were carried out using different molecular markers to evaluate genetic diversity in sweet potato genotypes (Zhang et al., 2000; Hu et al., 2003; He et al., 2005; Zhou et al., 2005; Qiang et al., 2009; Moulin et al., 2012). Zhang et al. (2000) found that genotypes collected from two different countries possessed different genetic background. Hu et al. (2003) used ISSRs for DNA fingerprinting of sweet potato germplasm. Zhou et al. (2005) used RAPDs for identification and selection of diverse parents for anti-nematode breeding. He et al. (2005) studied genetic diversity of 48 cultivars using 30 RAPDs, 14 ISSRs and 9 AFLPs in conjunction. Similarly, Moulin et al. (2012) also revealed genetic diversity among 81 cultivars using both RAPDs and ISSRs. Qiang et al. (2009) determined precise relationship among sweet potato cultivars on genetic basis as compared to agronomic traits, so that promising parental pairs could be identified and selected for breeding excellent quality edible cultivars.

Ginger

The molecular markers have more potential for identifying the plant relationship in ginger varieties than that of morphological markers due to its direct access to genetic material (Harisaranraj et al., 2009). Bardakci (2001) found that RAPDs have been effectively used for analysis of genetic diversity among clonal organisms. However, ISSRs are highly reproducible than RAPDs (Goulao and Oliveira, 2001). Very few studies have been conducted on the application of molecular markers to assess the genetic diversity in family *Zingiberaceae* (Palai and Rout, 2007; Sigrist et al., 2011). SSRs are considered as more efficient marker for genetic diversity analysis. Moreover, the development and characterization of SSRs would be useful for future studies assessing genetic diversity and genetic variance among turmeric germplasm (Sigrist et al., 2011). The applications of molecular markers could be helpful for plant breeders to find the new variations and explore the genetic factors that control hereditary quantitative traits. Thus, the development of huge set of molecular markers is required to assess the genetic diversity in ginger for crop improvement purposes.

Asparagus

Asparagus is a dioecious plant species mostly cultivated in desert areas due to its fine and good flavour. Few molecular studies have been conducted on genetic diversity of this species. Molecular markers i.e. RAPDs, SSRs and ISSRs have been used for genetic analysis of asparagus germplasm (Aceto et al., 2001; Aceto et al., 2003; Sica et al., 2005). Sica et al. (2005) used 23 ISSRs for analysis of genetic variations according to geographical origin for further crop improvement programs.

Ornamentals

DNA finger printing of different genotypes is an important activity of plant breeding programs. Varietal characterization is based on the phenotypic assessment of morphological attributes in elite genotypes which is insufficient to allow for variety description. In ornamentals, little research has been conducted regarding the genetic diversity using molecular markers. The achievements made in characterization of ornamental crops through molecular approaches are presented in Table 2.

Roses

Roses are most important cultivated ornamental plants in the world. Several studies were conducted using different types of molecular markers i.e. RFLPs, AFLPs, RAPDs, SSRs and ISSRs for analysis of genetic resources (Rajapakse et al., 2001; Reddy et al., 2002; Yan et al., 2005; Zhang et al., 2006; Jabbarzadeh et al., 2010). SSRs have been used for association of diploid and tetraploid genomic linkage maps, genetic diversity, phylogenetic studies and cultivar or hybrid identification in roses (Rajapakse et al., 2001; Zhang et al., 2006). Oyant et al. (2008) developed new SSRs for genome mapping of roses for different traits. Reddy et al. (2002) used ISSRs for precise and rapid characterization of highly diverse germplasm. Jabbarzadeh et al. (2010) used 9 ISSRs to find the relationship among seven species of roses. Several studies also proved useful in genetic mapping and QTL analysis for powdery mildew resistance in roses (Yan et al., 2005; Moghaddam et al., 2012).

Chrysanthemum

New cultivars are difficult to obtain in chrysanthemum through crossing due to self-incompatibility in the species, which may result in a high rate of failure in many crosses (Wolff and Rijn, 1993). Usually, new cultivars have been obtained by spontaneous mutations in vegetative reproduction (Schum, 2003). Induced mutations and somaclonal variations derived from tissue culture have also been used as new sources of variability (Zalewska et al., 2007). Genetic transformation plays an important role to obtain the genes of interest from chrysanthemum plants (Silva, 2003). Many studies have been conducted on the somaclonal variations in chrysanthemum, while most of them did not carry out molecular analysis of variants (Vilasini and Latipah, 2000). Molecular approach has been extensively employed in characterization of different chrysanthemum cultivars, derived mutants and their stability (Zalewska et al., 2007). Minano et al. (2009) reported molecular characterization and detection of somaclonal variation using RAPDs markers in eight cut flowers and two pot plant cultivars of chrysanthemum. In a previous study, Martin et al. (2002) assessed somaclonal variation in chrysanthemum cultivars and only one out of the four cultivars examined exhibited no variation (Martin et al., 2002). The high degree of variation ranged from 0.7% frequency in the cultivar Cascade Lavalloise

to 18.2% in the cultivar Brise Japonaise. Zhang et al. (2010) crossed two highly heterozygous chrysanthemum cultivars (Yuhualuoying and Aoyunhanxiao), and made an association map with combination of RAPDs, ISSRs and AFLPs markers on the basis of double pseudo test cross mapping approach.

Carnation

Different molecular markers have been used for assessment of genetic diversity, cultivar identification and mapping of plant chromosomes (Bornet et al., 2002). Wu et al. (2003) reported that RAPDs markers accurately distinguished relationship between wild species and cultivars of Chinese pink, and also demonstrated potential of molecular markers to support the programs of cross breeding in carnation. Evaluation of genetic diversity amongst inbred lines offers an excellent opportunity for selection of diverse parents in further breeding programs. Fu et al. (2008) used SRAPs, ISSRs and morphological markers for determination of genetic diversity between 22 inbred-lines of *D. chinensis*, one genotype of *D. barbatus* and one genotype of *D. superbus*. SRAPs markers were more efficient than ISSRs. Budak et al. (2004) found that SRAPs have higher capability for revealing polymorphic alleles than obtained using ISSRs.

Marigold

In marigold important traits can be identified using molecular markers, which might be used in breeding programs. RAPDs markers have been proved to be useful in genetic variability. The genetic variations among genotypes may possibly be due to out crossing nature of crop. Mor et al. (2008) used 25 RAPDs to evaluate the polymorphism based on DNA fingerprinting analysis of 9 marigold genotypes. Dendrogram indicated clear genotype and species differences, which confirmed the reliability of RAPD markers over protein electrophoresis. The genotype "French orange" was highly diverse from the rest of the genotypes.

Jatropha

Molecular markers have been used to describe the configurations of genomic variability among plant populations as well as to recognize the replicated accessions within germplasm collections. Jatropha has high level of variability due to cross pollination, which offers the breeder to start screening and selection of seed sources for desired traits (Ginwal et al., 2005). Assessment of genetic diversity and pedigree analysis were carried out through RAPDs and high level of genetic variations was recorded among the jatropha genotypes studied, which ranged from 0.00 to 1.00 Jaccard's coefficient (Subramanyam et al., 2009).

CONCLUSION

Evaluation of genetic diversity has become an international issue in vegetable and ornamental crops all over the world. So, molecular markers are very helpful for exploitation and management of genetic resources. Availability of genetic variations in crops is of vital importance for their further improvement by providing possibilities for the breeders to develop new and excellent cultivars or hybrids. Current study provides detailed properties and applications of molecular markers to determine genetic diversity on DNA basis in vegetable and ornamental crops. Conclusively, all molecular markers are very useful in the description of germplasm.

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