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Molecular Tools for Nursery Plant Production

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Abstract

Breeding strategies in nursery plants is lagging behind most of the agricultural crops while molecular methods have been adopted last decade. Identification and verification of varieties for nursery plants were applied by molecular tools. Marker assisted breeding utilizes the DNA markers linked to genes of interest to achieve efficient selection strategies. Marker assisted selection (MAS) is a process whereby a marker is used for indirect selection of genetic determinants of a trait of interest. There are different kinds of molecular markers, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites and single nucleotide polymorphisms (SNPs). These molecular markers allow high density DNA marker maps. In this review, all of these molecular markers have been applied widely among crops and ornamentals and the advantages and disadvantages have been listed. The best molecular markers are those that distinguish multiple alleles per locus (highly polymorphic) and are co-dominant.

Keywords: Nursery; Production; Ornamentals; Molecular markers; SSR; AFLP; RFLP; RAPD; SRAP

Introduction

Most of the traits of interest for plant breeding programs are quantitative traits. These traits are controlled by many genes and environmental factors. Phenotypic selection is the most common used form of selection in traditional genetic improvement programs. However, by using this method, you will not know which genes are actually being selected. With the development of molecular markers, marker assisted selection (MAS) become increasingly important in the coming years. MAS involve the selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers [1].

Molecular markers can be thought as constant landmarks in the plant genome. There are different kinds of molecular markers, such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites and single nucleotide polymorphisms (SNPs). These molecular markers allow high density DNA marker maps.

There are three types of relationships between the markers and the genes of interest. First, the molecular marker is located within the gene of interest. Second, the marker is in linkage disequilibrium (LD) with gene of interest throughout the whole population. Third, the marker is not in linkage disequilibrium with gene of interest throughout the whole population [2]. This study will give a general review about molecular markers used in nursery plants.

Molecular markers

In genetics, a molecular marker is a fragment of DNA that associated with a certain location within the genome. Molecular markers are usually phenotypically neutral and could identify by techniques such as southern hybridization or PCR. Several different kinds of molecular marker could be applied on plant selection: such as restriction fragment length polymorphisms (RFLPs), is detected by southern hybridization. The principle of RFLPs is detecting a site in a genome where the distance between two restriction sites varies among different individuals. These sites are identified by restriction enzyme digests of chromosomal DNA. It requires a radioactive probe when do southern blotting.

Other methods involve using PCR, such as amplified fragment length polymorphisms (AFLPs) uses restriction enzymes to digest genomic DNA [3]. Usually this technique has three steps: first, digestion of total plant DNA with one or more restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments. Second, selective amplification of a subset of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences. Third, run the amplicons on a gel matrix, followed by visualization of the band pattern. Random amplified polymorphic DNA (RAPDs) markers are about 10 nucleotide length DNA fragments from PCR amplification of random segments of genomic DNA. RAPDs are able to differentiate between genetically distinct individuals. In recent years, RAPD has been used to characterize the phylogeny of diverse plant and animal species [4]. Single nucleotide polymorphisms (SNPs) refer to a single nucleotide difference in the sequence of a gene or segment of the genome [5]. There are a variety of methods for analyzing SNPs; detection of SNPs can be done without gels, such as high resolution melting method. All of the above molecular markers have been applied widely among crops and ornamentals and the advantages and disadvantages have been listed in Table 1 [6]. The best molecular markers are those that distinguish multiple alleles per locus (highly polymorphic) and are co-dominant (each allele can be observed).

Sequence-related amplified polymorphism (SRAP) is a simple marker technique aimed for the amplification of open reading frames. Based on two-primer amplification, SRAP combines simplicity, reliability, moderate throughput ratio and facile sequencing of selected bands [7].

Current status of applications of molecular markers in nursery plants production

Molecular marker technologies have been widely used in

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Molecular marker	Advantages	Disadvantages	Codominant (C) or Dominant (D)
Amplified fragment length Polymorphism (AFLP)	Multiple loci High levels of polymorphism generated	Large amounts of DNA required Complicated methodology	D
Simple sequence repeats (SSRs) or microsatellites	Technically simple Robust and reliable Transferable between population	Large amounts of time and labor required for production of primers Usually require polyacrylamide electrophoresis	C
Restriction fragment length polymorphism (RFLP)	Robust Reliable Transferable across populations	Time-consuming, laborious and expensive Large amount of DNA required Limited polymorphism	C
Random amplified polymorphic DNA (RAPD)	Quick and simple Inexpensive Multiple loci from a single primer possible Small amounts of DNA required	Problems with reproducibility Generally not transferable	D
Sequence-related amplified polymorphism (SRAP)	Simple Reliable Moderate throughput ratio Facile sequence of related bands	Time and labor required for production of primers	C

Table 1: Advantages and disadvantages of most commonly-used DNA markers.

Ornamental	Trait	Samples	Methods	Primers	Gene/QTL	Linked marker	Year	Reference
Capsicum annuum L	Erect versus pendant orientated fruit	108 F2:3 individuals	Bulked segregant analysis (BSA) and amplified fragment length polymorphism (AFLP)		Saengryeog 211 (pendant), Saengryeog 213 (erect)	A2C79	2008	[9]
Oil Palm	Genetic diversity	6 Cultivars	Simple sequence repeat (SSR)	20 SSR markers			2012	[10]
Mei (Prunus mume Sieb. Et Zucc.)	Genome-wide characterization and linkage mapping	mei genome	Genome-wide characterization of simple sequence repeats (ssrs)	188,149 ssrs occurring at a frequency of 794 SSR/Mb.			2013	[11]
Ornamental kale (Brassica oleracea L. Var. Acephala)	Artistic diversiform leaf color	500 F2 individuals	Sequence related amplified polymorphism (SRAP)		Re (red leaf)	Me8Em4 Me8Em17Me9Em11	2013	[12]
Cherry plum (myrobalan plum)	Resistance to root-knot nematodes (RKN)				Ma1 and Ma3	SCAL19690 and SCAFLP2202	2004	[13]
Paeonia	Genetic diversity	29 cultivars	Sequence related amplified polymorphism (SRAP)	24 primers		Me8/Em8 Me8/Em1	2008	[14]
Dendrobium (Orchidaceae)	Genetic diversity	31 Chinese Dendrobium species	Sequence-related amplified polymorphism (SRAP)	14 primers	727 loci		2013	[15]
Aechmea gomosepala	Genetic divergence of bromeliad hybrids		Sequence related amplified polymorphism (SRAP)	16 primers	265 loci		2012	[16]

Table 2: Selected examples of gene-marker associated for important traits in ornamentals.

Ornamentals	Trait	Samples	Primers	Year	Reference
Heather (Calluna vulgaris)	Genetic mapping of the "bud-flowering"	Single mapping population	535 AFLP markers	2013	[17]
Evergreen azalea	Genetic diversity	130 genotypes	3 primers (408 polymorphic fragments)	2013	[18]
Mei (Prunus mume Sieb.et Zucc.)	Genetic diversity	65 accessions	64 -primer combination	2012	[11]
Sinningia speciosa	Genetic diversity	24 accessions of S. Speciosa	16 primers	2012	[19]
Viburnum	Interspecific cross		5 primers	2012	[20]
Sacred lotus	Genetic diversity	58 accessions	20 primers	2012	[21]
Spring orchid (Cymbidium goeringii)	Genetic diversity	Two wild populations	15 primer sets	2011	[22]
Aquilegia (Ranunculaceae)	Genetic diversity	64 accessions	16 primers	2011	[23]
Viola suavis	Parallel evolution of white-flowered morphotypes	36 populations	3 primers	2008	[24]
Berberis thunbergii	Influence of invasive populations	85 plants representing five invasive populations.	6 primers	2008	[25]
Ginkgo biloba	Genetic diversity	21 cultivars	64 primers	2006	[26]
Yellow camellia (Camellia nitidissima)	Genetic diversity	6 populations	8 primers	2006	[27]
Aglaonema	Genetic diversity	54 culivars	53 primers	2004	[28]

Table 3: Selected examples of amplified fragment length polymorphisms (AFLPs) marker assisted selection in ornamentals.

ornamental plants. Most of the traits of ornamental importance are quantitative traits with complex inheritance and regulated by several genes, the environment and their interactions. Moreover, improving polygenic traits through MAS is a complex process [8]. Because more than one gene is involved in a quantitative trait, these genes have smaller individual effects on the phenotype. So the effect of the individual genes cannot be easily identified. In the following tables, the reader can find a brief summary of the current status regarding application of MAS in the different ornamentals. Gene-markers associated for important traits in ornamentals are listed in Table 2. Furthermore, marker selections in ornamentals by using amplified fragment length polymorphisms (AFLPs) method are showed in Table 3.

Conclusions

In nursery plants production, the majority of application of molecular marker is used for genetic diversity studies. However, MAS for quantitative traits is a difficult task in ornamentals, as with many other crops. Further advances in molecular technology and genome programs will soon create a wealth of information that can be exploited for the genetic improvement of ornamental crops. High-throughput genotyping, for example, will allow direct selection on marker information based on population-wide LD. Methods to effectively analyze and use this information in selection are still to be developed. The eventual application of these technologies in practical breeding programs will be on the basis of economic grounds, which, along with cost-effective technology, will require further evidence of predictable and sustainable genetic advances using MAS. Until complex traits can be fully dissected, the application of MAS will be limited to genes of moderate-to large effect and to applications that do not endanger the response to conventional selection. Until then, observable phenotype will remain an important component of genetic improvement programs, because it takes account of the collective effect of all genes.

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