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USE OF MOLECULAR MARKERS IN DISEASE RESISTANT VEGETABLE BREEDING

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ABSTRACT

Today's vegetable breeding studies are primarily aimed at developing resistant varieties. The development of resistant varieties is obtained as a result of long breeding studies. Morphological determinants used in the determination of resistant genotypes in classical breeding studies help to differentiate genotypes but may be affected by environmental conditions. Homozygous and heterozygous individuals cannot be detected if any of these characters are recessive. Therefore, in recent years, advances in the use of molecular markers in the field of biotechnology in disease resistance have rapidly gained importance in the use of plant breeding. Molecular marker assisted selection (MAS) involves selection of plants carrying these genomic regions by means of molecular markers expressing the desired properties. Thus, saving time and space in the selection of disease-resistant varieties, hundreds of plants can be selected simultaneously and reliably and quickly. In this study, types, advantage-disadvantages and application areas of molecular markers used in selection of disease resistant varieties and/or parents in vegetable breeding are discussed.

1. INTRODUCTION

In our country, many chemicals are widely used in the control against disease both in open and greenhouse vegetable growing areas (Kaygusuz & Biçici, 2007; Hwang & Kim, 1995; Çolak & Biçici, 2013; Foster & Hausbeck, 2010). Although some effective chemicals have been developed for many plant diseases, some important diseases are soil-borne diseases such as Fusarium spp, Rhizoctonia spp., Phythium spp. and the disease may develop chemical resistance due to differences in inoculum density in the soil, and no effective chemical control can be achieved economically (Bowers & Mitchell, 1991; Parra & Ristaino, 1998; Walker & Bosland, 1999; Hwang & Kim, 1995). In parallel with the studies in the world, alternative approaches including organic and integrated agricultural systems have come to the fore in order to reduce the negative effects of chemical control in our country (Aksoy, 1999). Among the alternative agricultural systems, cultural measures take the first place in the control against plant diseases. Among these cultural measures, the use of resistant varieties is the most effective, most economical, environmental and sustainable method in the control against disease.

Today's breeding studies are primarily aimed at developing resistant varieties. The development of resistant varieties is obtained as a result of long breeding studies. Resistant varieties are mostly produced as a result of resistant wild species and crosses with existing varieties in order to have quality characteristics such as good taste, shape and color as well as resistance to a certain disease (Scott, 2005). Morphological markers used in classical breeding studies help to differentiate genotypes, but may be affected by environmental conditions. Homozygous and heterozygous individuals cannot be detected if any of these characters are recessive. Therefore, in recent years, advances in the use of molecular markers in the field of biotechnology in disease resistance have rapidly gained importance in the use of plant breeding. Thus, saving time and space in the selection of disease-resistant varieties, hundreds of plants can be selected simultaneously and reliably and quickly (Sambrook et al., 1989; Darling & Brickell, 1994; Summerel et al., 2001; Barone et al., 2005). In this study, types of molecular markers, usage areas in vegetable breeding and some molecular studies on resistance to diseases are mentioned.

2. MATERIAL AND METHODS

Breeding of varieties resistant to one or more vegetable diseases and pests is an alternative approach that limits the environmental and consumer risks of chemical applications. Molecular marker assisted selection (MAS); and selection of plants carrying these genomic regions by means of molecular markers expressing the desired properties. In MAS, DNA-based markers can be used effectively for monitoring the appropriate allele (dominant or recessive), and the selection of the most appropriate individuals for offspring selection is based on the allelic composition throughout a part or the entire genome (Morid et al., 2012). There are basically two different DNA marker techniques. One is RFLP (Restriction Fragment Length Polymorphism) based on DNA hybridization, the other is RAPR (Random Amplified Polymorphic DNA) based on PCR (AFLP), AFLP (Simple Sequence Repeats), SSR (Sequence Related Amplified Polymorphism) techniques. (Staub et al., 1996; Althoff et al., 2007; Zabeau et al., 2007; Williams et al., 1990). Among these techniques, PCR (Polymerase chain reaction) is the most commonly used method (Darling & Brickell, 1994; Kuramae & Souza 2002; Hernandez, 2004). PCR is defined as the enzymatic expression of copies of a specific DNA fragment in in-vitro with short-chain oligonucleotide primers (Mulis, 1990;.Arda, 1995; Gülşen & Mutlu, 2005).

Markers that do not differ between genotypes are defined as monomorphic markers. Markers that differ between individuals of the same or different species are called polymorphic markers. Polymorphic markers are more useful than monomorphic ones because they identify differences. Polymorphic markers show dominant and Co-dominant characteristics, as they can be distinguished between homozygous and heterozygous. Using Co-dominant markers, the difference between heterozygous and homozygous can be clearly seen. However, dominant markers cannot make this distinction. For this reason, Co-dominant markers are preferred in order to know from which parent (mother or father) the disease resistance gene comes from in vegetable breeding (Williams et al., 1990; Staub et al., 1996; Gülşen & Mutlu, 2005; Mutlu et al., 2008).

RFLP (Restriction Fragment Length Polymorphism): It is the first non-PCR based marker system developed. RFLP markers are Co-dominant. With this feature it is possible to identify heterozygous individuals. RFLP is the first molecular marker technique. It is used in phylogenetic studies with RFLP markers, taxonomy and gene mapping studies. RFLP markers, Co-dominated, high polymorphism and high reproducibility, as well as the need for quality DNA, more labor, time and expensive are among the disadvantages of the system (Staub et al., 1996; Bark & Havey, 1995).

RAPD (Random Amplified polymorphic DNA): PCR based, short oligonucleotide primers (6-10 bases) to determine the difference between genotypes. RAPD markers are an advantageous technique in terms of low DNA requirement, high polymorphism rate, and cheap and low labor requirements. However, because RAPD markers are dominant markers, it is difficult to interpret and low reproducibility of the results are among the major disadvantages (Williams et al., 1990).

AFLP (Amplified Fragment Length Polymorphism): AFLP technique is developed by utilizing the principles of RAPD-PCR method. In this method, the DNAs of the genotypes obtained as a result of the breeding are cut with two restriction enzymes (4 and 6 bases) and synthetic DNAs called adapter (ligation) are added to the ends of the DNAs. Ligation products are amplified using primers with selective nucleotide added and the number of selective nucleotides ranges from one to three. AFLP- PCR products are run on polyacrylamide gel and the results are evaluated according to the polymorphism. AFLP technique is between RAPD and RFLP in terms of highly reproducible properties, number of polymorphic bands, cost, labor and reliability. AFLP technique, 30-150 regions can be identified in a single reaction, determination of intra-species and interspecific kinship is an advantageous method in terms of reproducibility. The disadvantage is that the AFLP technique is an expensive and dominant marker (Vos et al., 1995).

SSR (Simple Sequence Repeats): It consists of 2-6 nucleotide sequences that are frequently repeated throughout eukaryotic genomes. The most common in SSR sequencing are dinucleotides, trinucleotides and tetranucleotides. The number of times these sequences are repeated in the genome studied and the location of these sequences differ from species to species. Primers specific to these recurrent regions have been developed in SSR. Thus, different alleles in a locus can be detected by PCR. SSR technique is highly polymorphic, relatively easy, Co-dominate marker and reproducible are important advantages. Especially in plants genetic mapping SSR technique is used successfully. The most important disadvantage of SSR technique is that it requires genome information in new marker development. In the SSR technique, the development of initiator DNAs for the identification and replication of nucleotide sequences is expensive and labor intensive (Guilford, 1997; Weber & May, 1989).

SRAP (Sequence Related Amplified Polymorphism): SRAP is a PCR based marker system targeting open reading zones.The SRAP technique directly amplifies gene regions. The size of the obtained bands can vary between 100-1000 bp. In the RAP technique, the bands are obtained by using forward and reverse primers of 17 or 18 bp length respectively. The SRAP technique is simple, inexpensive, has a high polymorphism rate, is suitable for gene labeling and cDNA fingerprinting studies, has the ease of sequencing the selected bands. SSR technique is used in genetic mapping, gene labeling and genetic diversity studies in different plant species. SRAP markers produce higher consistent results than RAPD markers. Compared to AFLP markers, it is cheaper and requires less labor (Li & Quiros, 2001).

CAPS (Cleaved Amplified Polymorphic Sequence): In the CAPS technique, specific DNA fragments (19-24 mer oligonucleotide primers) specific to the genome of the organism studied are sequenced to form primers specific to them. PCR is performed with these primers. PCR products are cut by cutting enzymes and differences are detected by separating them in agarose gel (Jarvis et al., 1994). CAPS markers can easily distinguish between Co-dominant and homozygous-heterozygous alleles (Konieczny & Ausubel, 1993). The advantages of the CAPS method include the need for very small amounts of DNA, the ability to discriminate from Co-dominant alleles, and simple and inexpensive. However, CAPS is widely used in gene mapping studies and molecular identification studies (Weiland & Yu, 2003).

SCAR (Sequence Characterized Amplified Region**):** In this method, DNA bands obtained from RAPD are cloned and DNA base sequences are determined. Specific primers of 18-24 base length are designed on a portion of this differing DNA fragment and utilized in the PCR study.The SCAR method is being used for screening genome libraries and gene mapping studies (Weising et al., 1995).

2.1. Desired properties of molecular markers

Molecular markers have many advantages over morphological and biochemical markers. Molecular markers; Because they are genome-dependent, they are reliable, reproducible, easy to analyze and simple, suitable for automation, and easily optimized between laboratories. They are also unaffected by environmental and genetic factors. In this context, among the most important features desired in the molecular marker; high polymorphic behavior and different genotypes. It should also show Co-dominant inheritance. Thus, heterozygous individuals and homozygous dominant individuals should be easily distinguished. Molecular markers must be uniform in the genome, reproducible, and require a small amount of DNA or tissue. The application cost should be low and easy to reach, the results should be evaluated easily and quickly (Gülşen & Mutlu, 2005; Helentjaris et al., 1985; Kesawat & Das, 2009; Williams et al., 1990).

2.3. Usage Areas of Molecular Markers

Molecular markers are used to determine the genetic resources of plant and plant diseases and to determine the kinship levels, to protect the varieties genetically and to protect certain varieties at the genome level. Nowadays, in many vegetable species, both greenhouse and field, molecular markers are used in a very short time and reliably to detect diseases and pests that cause economic damage. It is successfully used in breeding programs for the development of new varieties resistant to diseases and pests, genetic mapping, marker assisted selection studies, determination of genetic diversity within and between populations (Khan & Spor, 2001; Gülşen & Mutlu, 2005; Williams et al., 1990).

3. MOLECULAR MARKER ASSISTED SELECTION (MAS) STUDIES IN DISEASE RESISTANCE VEGETABLE BREEDING STUDIES

The main objective of vegetable breeding is to develop high quality and plant disease-pest resistant hybrid varieties. In this context, resistance selection with molecular marker selection (MAS) accelerates vegetable breeding studies, saves time and space in the selection of hybridizations made with the selection of desired genotypes and provides selection of hundreds of plants in one day (Staniaszek et al., 2007, Barone et al., 2005; Gülşen & Mutlu, 2005). It is important for the reliability of the results that the primers used in breeding of disease resistance selection with MAS are preferred to the closest gene. The use of SCAR markers in breeding compared to other SRAP (Budak et al., 2004), RGA (Mutlu et al., 2006), RAPD (Toppino et al., 2004; Boyacı & Abak, 2008) markers; breeding studies in the F2 and F3 populations homozygous or heterozygote in the determination of heredity is very close to the gene is very reliable, accurate and easily detectable in agarose gel quality (Mutlu

et al., 2008). In breeding studies, by selecting genotypes suitable for MAS selection purpose; It contributes to the rapid commercialization of lines, from time to saving, to lower costs in hybridization programs.

Pepper crown blight (*Phytophthora capsici* Leon) is one of the most important diseases causing loss of yield and limiting cultivation in all areas of pepper production in the world. Molecular marker selection provides many advantages in plant breeding studies, particularly in determining polygenic characters such as *Phytophthora capsici* Leon whose resistance is directed by many genes.This quantitative resistance is considered a promising tool for breeding (Thabuis et al. 2004).Quirin et al. (2005) in their study; Using the OpD04.717 primer, they obtained a single band in *Capsicum annuum* and *Capsicum chinense* species resistant to the causative agent. They cloned this band and sequenced it to form a marker.In this study, Study; researchers found that the marker they obtained was very close to the Phyto 5.2. on the 5. chromosome, one of the six QTLs providing resistance. They reported that it can be used to differentiate resistant individuals with Phyto 5.2. The disease resistance is a multiple gene-controlled character. However, important QTLs can be effective in making the control against disease sustainable. In this study; one of the QTLs that are resistant to *Phytophthora capsici* Leon has been mapped and converted to PCR based marker and presented to the use of breeding programs. (Quirin et al., 2005).

They studied the PCR-based RAPD and AFLP markers for resistance to powdery mildew (*Oidium lycopersicum*) expressed by ol-2 gene in tomato. The F2 population was formed by hybridizing with susceptible to powdery mildew Super Marmande variety and resistant *Lycopersicon esculentum* var. *cerasiforme.* At the end of the study, the RAPD marker $OPU3_{1500}$ was detected in susceptible bulk at 1500 bp. The RAPD marker was converted to CAPS and used for molecular marker assisted selection (MAS) (Giovanni et al., 2004). In China; investigated the inheritance analysis of the linkage of Late Blight of tomato (*Phytophthora infestans*) resistance to SSR (simple sequence repeat) markers, an important disease in tomato productionin China. In this study where heredity was defined; 241 F_2 tomato plants were obtained from the hybrid CLN2037E hybridization with 5 precision lines. As a result of genetic mapping and linkage analysis; the disease was controlled by the *Ph*-ROL gene on chromosome 9. The SSR marker TOM236 with the *Ph*-ROL gene has been reported to be at a distance of 5.7 cM (Zhu Shan et al., 2006).

Eggplant (*Solanum melongena*) cultivation; *Fusarium oxysporum* Schlecht. f. sp. *melongenae* Matuo and Ishigami (FOM), which causes wilt disease in plants, has been reported to cause significant yield losses in many countries such as Italy, Israel, Japan and the Netherlands, and damage both in field production and greenhouse cultivation (Kenneth et al., 1970; Capelli et al., 1995; Çolak et al., 2018). Fusarium wilt (*Fusarium oxysporum* f. sp. *melongenae*, FOM) resistance in eggplant in many studies; Fusarium wilt resistant wild species have been reported, including *Solanum indicum, S. integrifolium, S. aethiopicum gr. gilo, S. sisymbriifolium, S. incanum* and *S. torvum (*Mochizuki et al., 1997, Kashyab et al., 2003; Rotino et al., 2004; Toppino et al., 2008). Mutlu et al. (2008) developed that SRAP, SRAP-RGA, RAPD and SCAR markers to determine the resistance to Fusarium wilt (*Fusarium oxysporum* Schlecht . f. sp. *melongenae*) in eggplant. In the study, as a result of 2,316 primer combinations, the three markers were found to be closest to 2.6 cM. Co-dominant SARP marker Me8/Em5 and dominant SRAP-RGA marker Em12 / GLPL2 were found to be linked to the resistance gene by 1.2 cM, but RAPD marker H12 was also associated with 2.6 cM on two alleles. In the study, SRAP marker F2 and back hybrid 3 generations were found to be associated with the endurance gene and converted to two dominant SCAR markers. Two SCAR markers designed with this study may be useful in MAS selection breeding studies to determine Fusarium wilt resistance in eggplant. Çolak et al. (2018) in their study, resistance of 77 different eggplant genotypes against Fusarium wilt (*Fusarium oxysporum* f.sp. *melongenae*-FOM), *Potato Y potyvirus* (PVY) and Root-knot nematode (*Melodiogyne incognita*-RN) were determined with molecular marker assisted selection and classical testing. To determine FOM resistance, 77 eggplant genotypes were screened with the SCAR426 marker, which was identified to be the closest to the gene at 1.2 cM, and it was determined that the four eggplant genotypes, namely P11, P29, P49 and P52 were heterozygous resistant against FOM. FOM-resistant lines were verified by classical testing. As a result of classical testing conducted to determine PVY and RN resistance of eggplant genotypes, it was determined that all lines were PVY-sensitive and P29 and P52 genotypes were resistant to FOM and RN. Disease-resistant genotypes determined in the present study would contribute to the development of the new F1 hybrid eggplant cultivar.

The crown and root rot disease (*Fusarium oxysporum* f. sp. *radicis-lycopersici* / Frl), which is one of the most important soil-borne diseases that limit the production of tomatoes economically, is very difficult because of its soil origin (Çolak & Biçici, 2013). In molecular studies related to resistance to this disease, Truong et al. (2011) developed that the RAPD marker and transformed it into SCAR and presented it to its use in breeding studies. Mutlu et al. (2015) in their study; By transforming the SCAR marker into Co-dominant SCAR, they developed the $SCAR_{frl}$ marker, which can show whether the inheritance is heterozygous or homozygous at a distance of

approximately 0.016 cm at a distance almost above the Frl gene. Çolak et al. (2019) in their study; RAPD primer and SCARFr1 primer were used to determine FRL resistance, CAPS and Co-dominant SCAR primers were used for resistance to *Tomato yellow leaf curling virus* (TYLCV, Israel, Mild, Sardinian strains). In the 418 tomato genotypes; 102 of the FORL, 46 of the TY3 and 35 of the TY1 locus were determined resistance. In this study, 7 tomato genotypes which contain all three targeted gene sources (FORL + TY1 + TY3) were determined as a result of molecular studies and and classical tests.

Molecular marker-based resistance to many vegetable diseases studies; It is emphasized that it is important to conduct validation studies with classical testing at every stage of hybridization programs.Thus, Mutlu et al. (2008) 's Fusarium wilt resistance in eggplant in their study; Although such markers, such as SCAR426 and SCAR347, are very close to the gene and the recombination rate is very low, they report that it is necessary to verify disease resistance by classical testing at regular intervals within the hybridization programs of breeding materials determined to be resistant by markers (Scott et al., 2015).

4. CONCLUSIONS

In today's conditions, resistance to at least 3 and more disease factors is needed depending on the production area and time. As the required number of durability increases, the reclamation time increases and is not even possible. In this context; In recent years, rapid advances in the use of markers in molecular marker assisted selection (MAS) in the field of biotechnology in disease resistance have rapidly gained importance in the use of plant breeding. Thus, saving time and space in the selection of disease resistant varieties can be provided, hundreds of plants at the same time, can be selected safely and quickly (Darling & Brickell, 1994; Barone et al.,2005). Although molecular markers provide great advantages in breeding in terms of speed and cost, conventional validation tests must be performed in disease resistant genotypes (Lee et al., 2015). Because the distance of the developed markers again, taking into account the margins of human and marker origin, it is important to carry out classical validation tests in determining the resistance to MAS selective diseases (Caro et al., 2015; Scott et al., 2015).

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