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# Molecular Marker Technology: Current State and Prospective Directions (Review)

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**The article provides detailed information on molecular marker technologies that are widely used in various fundamental and applied fields of science. Characteristics of the majority of existing types of molecular markers, their advantages and disadvantages are given. By being part of the genome, molecular markers carry a lot of information about its changes during ontogenesis and phylogenesis. This circumstance turns them into an excellent tool in studying evolution, systematics, genetic and epigenetic mechanisms of adaptation, as well as in creating more stress-resistant forms, in genetic mapping, genetic tagging, "genome editing", determining the quality of the surrounding environment, addressing issues of eco - and genotoxicology, diagnostics, forensic-medical expertise, etc. The results of numerous studies in which Hybridization or PCR-based molecular markers used for some of the above-mentioned purposes have been discussed as well.**

***Keywords:*** *Molecular marker, restriction fragment, polymorphism, amplification, cpSSRs, ESTs*

## INTRODUCTİON

Biotechnology is one of the scientific areas open to the novelty. Molecular marker technologies - a practical achievement of this field - are very important both in scientific and practical terms. Development of these technologies started recently and within a few decades they began playing a great role in solving the problems of fundamental and applied sciences. There have been developed of many types of molecular markers, and combined with sequencing technologies, they aim to improve diagnosis, increase yields, etc. Progress in areas such as molecular selection, molecular genetics, genomic selection, genome editing has contributed to a more thorough understanding of molecular markers, and providing their greatest variety. Molecular markers are one of the powerful tools for analyzing genomes and allow us to establish the relationship between hereditary characteristics and the underlying genomic variability. Genotyping based on next-generation sequencing technologies contributes to the development of new markers for complex and unstructured populations.

The first markers, of course, were classical and, in the first place, morphological ones, according to which the state of the organism, in particular, plants, is evaluated. The following classical, no less informative and widely used, along with modern molecular ones, are cytological, biochemical markers. A molecular marker is usually called some gene in the genome or some region in the gene belonging to some fragment of DNA , a molecular marker is a nucleotide sequence associated with a trait of interest.

Molecular or DNA markers are classified based on different criteria. These criteria can be:

- a method of detection (on this basis they are divided into hybridization-based (historically the first) and based on PCR (Hybridization-based and PCR-based);
- the nature of the action on genes (dominant and co-dominant);
- the method of transmission to the offspring (on the paternal line by organoids, on the maternal line by organoids, by the nuclei of both parents, on the maternal line by the nucleus).

RFLP is the first and only method developed on the basis of hybridization. After the discovery of PCR, a large number of molecular marker methods that are widely used have been developed. These marker technologies are effectively used in diverse areas such as genetic diversity analysis, the design of genetic maps, genetic tagging, cloning, the creation of more stress-resistant variations, the analysis of the genotoxicity of substances, the study of epigenetic mechanisms of adaptation both during ontogenesis and phylogenesis, the study of systematics of various taxa, phylogenetics, evolutionary genetics, etc.

Thus, all the existing diversity of molecular

marker technologies can be divided into 2 main groups (based on the method of detection):

- based on hybridization
- based on the PCR system.

RFLP (Restriction fragment length polymorphism) is the first and perhaps the only hybridization-based molecular marker system that has been extensively used at the beginning of the molecular biology era. However, hybrid systems such as microarrays and diversity array technology (DArT) are currently being intensively used for detection of one nucleotide polymorphism (SNP - single nucleotide polymorphisms). Conversely, a lot of molecular-marker detection methods based on PCR have been developed. For example, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and sequence related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), sequence tagged site (STS), and sequence characterized amplification region (SCAR) are mainly used for genomic analysis.

All molecular-marker systems have their advantages and disadvantages. For example, RFLP markers can be applied to closely related species and this is an advantage in comparative genomics. But in RFLP, the detection procedure is relatively complex and expensive, and therefore it is not easy to automate the procedure for thousands of individuals. AFLP is a widely used molecular marker system because it can detect multiple genetic loci in the genome and this is its advantage. On the other hand, there are many stages in the AFLP procedure that limit its use in marker assisted selection - when thousands of DNA samples need to be analyzed in a short time. SSR systems provide a high level of polymorphism in plant genomes and are commonly used in most genomic applications. Yet, the SSR technology detects only repeats in the sequence, while the number of SSRs in the genome is relatively limited compared to the number of SNPs. RAPD is easily performed in one round of PCR, although the low reproductive rate of RAPD amplification limits its wide use in genomic assays, etc. (Nadeem et al., 2018)

Molecular-marker technology is ideal when it has the following characteristics:

1) polymorphism, which makes possible the use of the entire genome;

2) the ability to detect genetic variations;

3) the ability to apply many reliable and independent markers;

4) simplicity, speed and low cost of production of markers;

5) a small amount of DNA or tissue is required;

6) has such functions as correlation to various phenotypes.

But no molecular-marker technology has all these features at once. Therefore, in many cases, a combination of different markers is used. For example, Amar et al., (2017) presents the results of a comprehensive analysis of the genetic diversity of local and international garnet varieties in Egypt, based on ISTR, ISSR and SRAP markers profiles. The set of ISTR, ISSR and SRAP combinations of the primers was compared. The comparison was made according to the degree of resolution, the effectiveness of the discriminating ability of the method (along with the level of genetic polymorphism) in order to determine which varieties evolved from others, i.e. evolutionary relationships. During the study of the SRAP results, the analysis proved to be the best in almost all parameters and it gives much more evidence about the total number of effective alleles, the number of polymorphic amplicons, whereas ISTR markers showed an average level of polymorphism.

The genetic stability of soybean seedlings was studied under allopathic treatment of them, fallen and mixed with soil in a different ratio of eucalyptus leaves (Abdelmigid and Morsi, 2018). The combination of RAPD and ISSR-PCR markers showed that the DNA of such soybeans demonstrates a general tendency to decrease GTS (genetic template stability) and with increasing dose of exposure, a decrease in GTS occurs more. Low GTS of plants subjected to stress reflects their high genetic instability. The results allowed the authors to offer the most appropriate combination of RAPD and ISSR markers for determining the allopathic tolerance of Glycine max (soybean) and for application in breeding programs.

**1. Hybridization-based markers.**

**RFLP** (Restriction Fragment Length Polymorphysm).

The main stages of RFLP:

- obtaining a pure DNA preparation
- treatment of DNA preparations with restriction enzymes that cut a DNA molecule into certain loci (in recognition sites), resulting in a mixture of a large number of DNA fragments with different lengths
- separation of the mixture into separate fragments in EF (in agarose or PAGE gel).
- transfer of fragments from the gel to the membrane
- hybridization with radio-labeled DNA samples.

Fragments form a polymorphic bands in the EF-gel. Each band is a population of DNA molecules with the same length. The reason for such a diversity between the fragments of two DNA

preparations (for example, extracted from the cells of different individuals of the same species) can be the loss or addition of DNA sites (InDel mutations), or the change in recognition site by other mutations (point mutations, translocations, duplications, inversions etc.). As a result of such changes, new recognition sites for restriction enzymes are lost or, on the contrary, new ones are added, which in turn leads to polymorphism between fragments. Individuals of the same species may differ from each other, i.e. they can exhibit polymorphism. If the genome of the individual is heterozygous, polymorphism is manifested only in relation to one chromosome.

**2. PCR-based molecular technologies.**

There are many more of them. The efficiency and sensitivity in their implementation are mainly determined by the correctly chosen primer (annealing temperature, stability of the duplex primer-template, primer length, GC%, melting and annealing temperature of primer, etc.) (Williams et al., 1990; Jones et al., 1997). If all the PCR conduction rules are followed but the primer is not selected correctly, then the PCR protocol results in inadequate results. Thus, all PCR-based technologies, apart from everything else, depend on the correct choice of the primer.

**RAPD (randomly amplified polymorphic DNA).** RAPD technology was developed independently by two groups of scientists (Williams, et al 1990, Welsh and McClelland, 1990). In this method, DNA amplification occurs using a random, short and single-stranded primer. Amplicons fully depend on the size of target genome and primer (Jiang, 2013). The reproducibility of RAPD markers depends on such factors as the amount of DNA, PCR buffer, concentration of $MgCl_2$, annealing $t^o$, Tag-Polymerase, etc. (Wolff et al., 1993).

The influence of various chromium concentrations on the phenotypic growth characteristics of T.aestivum L. was studied (Rai and Dayal, 2016). The pollution of the environment by chromium compounds is relevant for all countries of the world, it occurs in connection with the development of the metal processing industry, the use of fungicides, the production of paints, cleaning of petroleum products, etc. Plants growing in contaminated areas and phenotypically altered were analyzed for the presence of polymorphism in PCR with RAPD primers. Based on the results, the authors came to the conclusion that, firstly, Cr at a concentration of 80 ppm is capable of causing a mutagenic effect in wheat, and secondly, several primers must be used to maximize detection of DNA changes.

RAPD markers can be used for early identification of plant tolerance. So, RAPD analysis was applied (Saleh, 2016) to assess DNA damage in 4 local varieties of cotton growing in non-saline and salt stress areas (200 mM NaCl). Changes in RAPD profiles are measured as genomic template stability (GST% = (1- a / n) x 100, where (a) is the average number of changes in the DNA profile and (n) the number of total bands in the control). As expected, the greatest changes occurred in two salt-sensitive, and the lowest - in 2 tolerant varieties.

The work of Olorunfemi et al. is an example of the use of molecular markers in eco-genotoxicology (Olorunfemi et al., 2015). In this study, RAPD analysis was used to assess the level of DNA damage in the cells of the root meristem of Allium cepa L. grown in contaminated drinking water. It turned out that in the DNA samples of plants that were watered with polluted waters, there were big changes in RAPD patterns. The authors suggest that the determined DNA polymorphism is caused by the genotoxic action of substances present in contaminated drinking water, which in turn can cause health problems. The results of such ecotoxicological studies are of great importance in genotoxicology

Altwaty and others studied the genotoxicity of one plant - D.glaucum, which is widely grown in Saudi Arabia - on another plant (Altwaty et al., 2016). The authors studied DNA polymorphism in a V. faba plant exposed to 3 types of Dipterygium glaucum extract (water, ethanol and ethyl acetate extracts) using RAPD markers and found the polymorphic effect.

RAPD markers in combination with STR markers were useful in detecting polymorphism in plants chronically irradiated with low doses of ionizing radiation (Khudaverdieva et al, 2005).

The sensitivity of lichens to air pollution is a well-known fact. But a detailed analysis of DNA with RAPD markers (Cansaran-Duman et al., 2015) made it possible to uniquely identify the most sensitive bioindicator of pollution (sulfur dioxide, nitrogen oxides, PM, generated by low-quality lignite used as fuel for household heating, and also released into the atmosphere by motor transport) of atmospheric air. P.praetextata proved to be the most sensitive species among the 4 investigated and the authors suggested using this species as a bioindicator of the environmental quality. It is proved that in order to obtain a reliable estimatation of the effects (Abdelhaliem and Al-Huqail, 2016), various methods (isozyme analysis and RAPD-PCR, as well as molecular cytogenetic assays such as single-cell gel electrophoresis-comet assay) for the analysis of genotoxicity of environmental pollutants should be applied in a comprehensive manner. RAPD-PCR and comet assay are the two

qualitative and quantitative methods that have been used to detect DNA damage and mutation. The authors studied the genetic effects of elevated levels of $CO_2$ and $O_3$ (alone or in combination) on wheat proteins and DNA (*Triticum aestivum* L.) under irrigated and non-irrigated conditions.

**AFLP markers.** Was developed by Vos P. et al. (Vos et al., 1995).

The sequence of procedures in this PCR technology is as follows:

1) restriction of genome DNA by two restriction enzymes (EcoRI and MseI) with the formation of fragments with protruding 3 '- ends; 2) restriction fragments of DNA are ligated with oligonucleotide adapter containing "sticky" ends for these restriction sites; 3) two consecutive PCRs are then carried out: in the first PCR, there are used primers that are fully complementary to the EcoRI and MseI adapters, therefore, a large number of amplicons are created between the EcoRI and MseI adapters, while EF separation is impossible. In the second PCR, primers are used, at the 3 ' – end of which there are bases that are non-complementary to adapters (1 to 3) and due to them selective amplification takes place. The Electrophoretic separation of DNA fragments is carried out in a denaturing PAAG gel.

AFLP are dominant markers, their polymorphism is higher than that of RAPD and ISSR. In this, as well as in an unlimited number of samples in each analysis, AFLP is superior to RAPD and ISSR. The method allows the specific co-amplification of high numbers of restriction fragments (typically 50-100 fragments). Expensive equipment, software and also expensive consumables are its drawback.

**SRAP (sequence related amplified polymorphism).** SRAP was developed by Li and Quiros, mainly to amplify open reading frames in 2001 (Li G and Quiros, 2001). This marker system is based on the use of 2 primers (Li G. et al., 2013). The initial idea of the authors was to simplify AFLP and improve productivity, as well as to improve reproductive performance compared to RAPD. To obtain a simplified detection procedure, the authors in the AFLP - detection protocol omitted the stage of restriction digestion and DNA ligation of target fragments. SRAP primers were created in size close to the primers AFLP, but instead of 2 cycles in AFLP, 1 cycle of PCR was applied. To detect multiple loci with a pair of SRAP primers, the authors invented a special PCR procedure program (94°C for 1 minute, 35°C for 1 minute, 72°C for 1 minute / first 5 cycles) and the next 30 cycles at an annealing temperature of 50°C. Relatively low annealing temperature (35°C) at the beginning of PCR allows the SRAP primers to be annealed to multiple loci in the target DNA, thereby multiple loci were amplified and profiles similar to those in the AFLP were obtained. Similar to AFLP, most SRAP markers are dominant. Unlike RAPD, SRAP uses a pair of primers of 16 and 22 nucleotides, instead of 10-nucleotide short primers. This is a great advantage of SRAP technology compared to the RAPD system, since 1 SRAP primer can be combined with an unlimited number of other primers. Although SRAP PCR starts with 35°C t$^{o}$-annealing in the first 5 cycles, larger sizes of SRAP primers allow increasing the annealing temperature up to 50°C in the following cycles, which greatly improves reproductive performance in SRAP. In addition, SRAP primers can be labeled with fluorescent labels and combined with untagged SRAP primers, so SRAP PCR products can be separated in capillary instruments such as ABI genetic analyzers.

It is known that there are differences in the GC content between the coding and non-coding sequences in the plant genome. These differences can be used in the development of two sets of SRAP primers. The forward primers containing the GGCC cassette closing the 3' end of SRAP primers that might preferentially anneal to the GC-rich regions while the reverse SRAP primer set was incorporated with an AATT cassette that would preferentially anneal SRAP could preferentially amplify gene-rich regions in a genome. After sequencing the SRAP fragments and constructing the SRAP of the B. oleracea genetic map, it was found that SRAP did indeed amplify the sequences from the genes, and a larger number of SRAP markers fell in the region of the chromosome arms, and less in the centromeric regions, which normally filled the AFLP markers.

Depending on the goal of the researcher, it is possible to design various SRAP primers, this is possible due to the wide flexibility of SRAP primers in development. To increase the capacity and effectiveness of SRAP technology, SRAP Illumina's Solexa sequencing can be combined to directly integrate genetic loci into the genetic map of *B.rapa*, based on paired-end Solexa sequences (W.Li et al., 2011).

Like multi-locus, SRAP markers have many advantages and it is an effective tool for conducting comparative genomics (Guo et al., 2012). In addition, SRAP markers do not require a complex of restriction enzymes and for pre-amplification, what is needed for AFLP, which makes SRAP a more effective molecular marker system for genome research.

SRAP - technology has several advantages for gene tagging, and for this feature it is superior to other molecular markers. The authors show that

they used SRAP technology for cloning and the characteristics of a gene that controls features such as color of the seed coat and hairiness in *B.rapa* (Long, Y. et al., 2010).

SRAP - technology is an effective molecular marker system also for qualitative and quantitative analysis of resistance to phytopathogens. Usually qualitative resistance is an oligogenic feature, and quantitative resistance is multigenic. Both the use of SRAP and other markers, as well as the correlation of results, have made it possible to identify the multi-gene character of resistance to many diseases in many plants (Chen et al., 2012; Li et al., 2012; Li et al., 2008; Mutlu et al., 2008; Tanhuanpaa et al., 2007; Yi et al., 2008).

Some authors have done an enormous job for the development of molecular markers associated with resistance genes to various factors (in this case, resistance to nematodes of cucumber root nodes (Meloidogyne spp) (Devran et al. 2011). They tested 100 AFLP markers and 112 SRAP (sequence related amplified polymorphism) markers. They used them to screen for resistant and susceptible parents for detecting polymorphism. Out of the 100 AFLP primers, 92 bands were synthesized, 2 of which were selected as putative candidate markers. These 2 strips of gel were transferred (eluted) to the solution, cloned and sequenced. The primers synthesized from these sequences did not give polymorphic bands between the parents. In these sections of DNA, there were no restriction sites to convert them to CARS or SCAR markers. Therefore, in the future SRAP primers and fragments developed from these primers AFLP became to be used to detect polymorphism between parents. Of the 112 primer combinations, 11 polymorphs were detected.

Molecular markers associated with resistance to root nematodes were also used in the other work (Ali et al., 2014). The authors investigated the inheritance, as well as the mapping of the mj-2 gene in carrots, the crop of which is severely affected by this pest (*M.javanica*), resulting in deformations of the carrot roots. And this reduces the productivity of agricultural products, especially in warm climates, which is very important for our region as well. Using the appropriate molecular markers associated with the mj gene, an arrangement was found for the so-called mj-2 gene on the same chromosome as mj-1 - previously described resistance gene to nematodes, but in another locus. It was found that the sign of resistance to root nematodes is controlled not by a single gene i.e. it is not formed under the control of one gene.

Genetic diversity was first studied among 13 species of Silene genus, growing in Iran, using SRAP markers (Bargish and Rahmani, 2016). This genus (Silene) is one of the most complex regarding taxonomy. With the use of 15 combinations of SRAP primers, 62 fragments were obtained, 71.9% out of which were polymorphic. The polymorphism varied in the range of 50-100%.

Consequently, SRAP markers are highly reproductive and optimal markers for evaluating genetic variation in clove (Silene). This and many other works show that molecular markers can be successfully applied in the analysis of genetic diversity. And the study of genetic relationships among plant taxa at the species and generic levels is of great importance, since this analysis gives information about the direction and the successive stages of plant evolution (Savolainen and Chase, 2003).

The bacterial wilt caused by Ralstonia Solanacearum is one of the diseases that causes huge damage to the potato yield. SRAP technology was used to detect loci that determine resistance to this pathogen (Yanping et al., 2014). In this work there were used easily cultivated, highly resistant species to produce F1 to carry out bulked segregant analysis (BSA) for screening and identifying SRAP markers associated with potato resistance to the bacterial wilt. A clutch map was developed for 23 DNA markers distributed over 3 clutch groups. The results of this work show that this marker system can be used in marker assisted selection (MAS) of plants.

SRAP markers are mainly used for agronomic purposes, developing in modern hybrids loci of quantitative traits and for assessing the genetic diversity of large germplasm collections. But some authors believe that SRAP markers should also be used to solve hypotheses in systematics, biogeography, conservation, plant ecology and others (Robarts and Wolfe, 2014).

The paper (Soleimani et al., 2012) gives the results of assessing genetic diversity using SRAP markers of 63-cultural, wild and decorative garnet genotypes, selected from 5 different geographical regions of Iran. The authors confirm with their results that SRAP markers can be powerful tools and an effective marker system for determining the genetic diversity and genetic structure of the pomegranate population.

**TRAP (targed region amplification polymorphism).** A rapid and efficient PCR-based target region amplification polymorphism (TRAP) technique was developed by Hu and Vick (Hu and Vick, 2003). TRAP markers are a PCR-based method by which a large number of loci can be determined (Barakat et al., 2013).This marker system is characterized by simplicity, high throughput, with numerous co-dominant markers, with high reproducibility (Alwala et al., 2006).

TRAP uses bioinformatics tools and the EST database information to generate polymorphic markers around targeted putative candidate gene sequences. Thus, it should be useful in plant genomics research in genetic mapping and marker-trait association (Liu et al., 2005). Previously, TRAP was also used to estimate the genetic diversity in the genetic stocks of wheat (Xu, Hu and Faris, 2003; Al-Doss et al., 2011).

In TRAP technology, 2 types of 18 nucleotides are used to create markers. One of the primers is stable, developed on the basis of EST bank data, and another primer is designed for AT- or GC-rich regions. Unlike the SRAP method, TRAP requires information about the cDNA or EST sequence to design a specific primer. Like SRAP markers, TRAP markers are used in the analysis of the genetic diversity of germplasm, in the construction of genetic maps (including the construction of transcriptome maps), genetic tagging of important features and in the cloning of genes in many crop plants (Hu et al., 2005; Chen et al., 2006).

In TRAP technology, almost the same number of markers as AFLP is created. But, it does not require extensive pre-PCR processing of templates, as does the AFLP technique. An evaluation of 60 clones of the economically important species, *Paullinia cupana* var. *sorbilis*, preserved in Active Germplasm Bank (BGA), was conducted using TRAP and SRAP markers (Elizangela Farias da Silva et al., 2016). The level of polymorphism was 79% when using TRAP - and 74.5% using SRAP-markers. Thus, the combination of 2 markers can expand the genetic characteristic and facilitate the selection of parental forms in breeding, what is also confirmed in other works (Menzo et al., 2013).

**ISSR (inter simple sequence repeat) markers.** This marker system was developed by Zietkiewicz et al. (Zietkiewicz et al., 1994). ISSRs makes it possible to determine polymorphism in intermicrosatellite loci (using primers of 2 or 3 nucleotide repeats). This method does not require information about the sequence of the genome and a high level of polymorphism can be realized (Chatterjee et al., 2004). ISSR is based on the amplification of DNA segments located between two identical but oppositely directed microsatellite repeats. The distance between these microsatellites should allow the flow of amplification. The primers used in this method-microsatellites can be di-, tri-, tetra- and penta-nucleotide repeats. In this method, high annealing t$^{o}$ (40-60°C) can be used, while amplicons have a length of 200-2000 bp (Fang and Roose, 1997, Moreno et al., 1998). Although ISSR markers are characterized as dominant, they can also be used in the development of co-dominant markers (Zietkiewicz et al., 1994; Tsumura et al., 1996; Ng and Tan, 2015). Relative low reproducibility is a disadvantage of these markers (Semagn et al., 2006).

The authors used several marker systems, in particular ISSR markers, to evaluate the effects of lead on DNA (Manfouz and Rayan, 2016). The main facts observed in the ISSR patterns of Hordeum vulgare, in the study of the effects of lead exposure, were the following: loss of bands that are present in the control, and the appearance of new ones that were not present in the control. Of the 9 used primers, almost all had more than one change and the maximum number of changes in the ISSR bands corresponded to a high concentration of lead (150 g / l). The authors explain the differences in the patterns by the presence of photoproducts (e.g. pyrimidine dimers) in the DNA, which could block or reduce polymerization of DNA in PCR. It is suggested that the DNA damage may be serious in the majority of the barley seedlings exposed to toxic chemicals. Apparently, at high lead concentrations, DNA damage is so great that DNA polymerase is more often blocked and as a result, the differences in PCR profiles are greater compared to other variants. The authors note that ISSRs are simpler among the molecular markers and this system is more adaptable, since their use does not require preliminary information on target sequences, they are effective and reproducible (Bornet and Branchard, 2001; Fang and Roose, 1997; Pradeep-Reddy et al., 2002).

Various calluses of the same parent cauliflower were analyzed by ISSR markers to characterize genetic instability (Xavier et al., 2000). Various ISSR markers exhibited a different degree of polymorphism. After sequencing, one sequence showed homology with the A.thaliana gene, closely associated with the mammalian genes involved in the regulation of cell proliferation. This marker is characterized by 3 microsatellites with palindromic sequence. Possible causes of mutations in this marker are discussed. ISSR amplification appears to be a reliable method for determining genetic instability at the early stages of in vitro culture. ISSR markers in combination with other markers are also used for genetic mapping (Chen et al., 2011).

With the help of ISSR technology (in combination with other molecular markers - ITS1 and ITS4, PCR-RFLP), it was possible to detect changes in the DNA methylation profile of maize under fractionated UV-C irradiation, and also to establish the relationship of these changes with the release of chromosomal aberrations (Kravets et al., 2013). This relationship was also previously reported (Hauser et al., 2011).

**MSAP (Methylation Sensitive Amplified Polymorphism) markers.** This method is designed

to assess the degree of methylation of cytosine and has been successfully applied to the genomes of many species (Arabidopsis, grape, maize, tomato, and pepper species). The method is based on the use of isoschizomers, which differ in their sensitivity to methylation. MSAP approach is used also to assess the extent of cytosine methylation under salinity stress (which is a major ecological and agronomical problem and this problem is more acute in regions where salt water is used for irrigation) in salinity-tolerant and salinity-sensitive rapeseed cultivars (Marconi et al., 2013). Data of this study show that salinity affected the level of DNA methylation (an increase of 16.8%). In particular, methylation decreased in salinity-tolerant and increased in salinity-sensitive cultivars. Nineteen DNA fragments showing polymorphisms related to differences in methylation were sequenced. In particular, two of these were highly similar to genes involved in stress responses and were chosen to further characterization. The authors concluded that plants can employ regulatory strategies, such as DNA methylation, to enable relatively rapid adaptation to new conditions.

Another example of the use of MSAP markers is a study conducted by Guangyuan et al., (Guangyuan et al., 2007). In this work, genetic damage and DNA methylation induced by salt stress in *B. napus L.* were evaluated by AFLP, SRAP and MSAP markers. In the MSAP analysis, 3 strips with significant deviations from other PCR bands were identified. Analysis of the results showed that after saline treatment, methylated CCGG sites increase by 0.2 -17.6%. Nine methylation sites were sequenced and 1 site identified with a high degree of homology with the ethylene responsive element binding factor (ERF) sequence. These results demonstrate clear genetic and epigenetic changes in plants in response to salt stress and these changes underlie the mechanism of plant adaptation to salt stress.

Two forms of sequence based marker, Simple Sequence Repeats (SSRs), also known as microsatellites, and Single Nucleotide Polymorphisms (SNPs) now predominate in modern genetic analysis. Reducing the cost of DNA sequencing has led to the availability of large sequence data sets derived from sequencing of whole genomes and to the detection of large scale EST (Expressed sequence Tag), which allow the development of SSRs and SNPs, and the latter in turn can be applied to diversity analysis, genetic trait mapping, association studies, and marker assisted selection. These markers are inexpensive and require minimal laboratory work, for the development.

**SSRs (Simple Sequence Repeats).** These markers are short tandem repeats and simple sequence length polymorphisms. SSRs are tandemly repeating 20 bp motifs from 1 to 6 nucleotides. SSRs are present in a large number in the genomes of various taxa (including those in prokaryotes) (Tautz, 1989; Schlotteröer et al., 1991). Microsatellites can be mono - (A), di - (GT), tri - (ATT), tetra - (ATCG), penta - (TAATC) and hexa - nucleotides (TGTGCA) (Weber, 1990). They occur in the nuclear and cytoplasmic (in mitochondria and chloroplasts) genomes (Rajendrakumar et al., 2007; Melotto-Passarin et al., 2011). There is evidence that SSRs exist within protein-coding genes, as well as in ESTs (Morgante et al., 2002; Ding et al., 2015). SSRs are codominant markers, have high reproducibility, provide a high level of polymorphism, can be used in studies on plant gene mapping (Kashi and King, 2006). This system requires a very small amount of DNA and there is the possibility of automating procedures. SSRs were initially considered evolutionarily neutral markers, but later there was obtained an evidence of their important role in the genome evolution (Moxon and Wills, 1999). It is believed that SSRs are involved in the expression, regulation and functioning of genes, they are of functional importance even in noncoding regions of the gene (Kashi et al., 1997; Li et al., 2002; Mortimer et al., 2005).

**cpSSRs (chloroplast microsatellites).** The chloroplast genome is characterized by a low mutation rate and therefore it is difficult to detect enough sequence variations. Conversely, cpSSRs provide a high degree of polymorphism, due to which they are very useful in studies on population genetics (Provan et al., 2001; Melotto-Passarin et al., 2011). cpSSRs are mononucleotide motifs that repeat 8 to 15 times. The level of polymorphism in cpSSRs is quite variable within the species and locus. cpSSRs differ from nuclear microsatellites by such properties as single-parent inheritance and non-recombinantity of the molecule. They have found wide application in both fundamental and practical fields of plant science (Park et al., 2016; Gregory et al., 2014).

**Mitochondrial microsatellites (mtSSRs).** mtSSRs of plants are highly dynamic, the greatest and smallest density of genes are present namely in mtDNA. Their sizes range from 200 to 2500 bp and contain various repeating elements and introns (Liu et al., 2011). mtDNA - markers are characterized by a low rate of evolution. The use of these markers is mainly limited to population genetics (Duran et al., 2009).

**SNPs (single nucleotide polymorphism).** SNPs markers are the most abundant source of genetic polymorphism, represent single nucleotide

differences in a specific DNA location of two individuals. There are 3 different categories of SNPs: transitions - C / T or G / A, transversions (C / G, A / T, C / A or T / G) and small inserts / deletes (InDels). SNPs in certain places can be bi-, tri-, or tetra-allelic, although tri- and tetra-allelic SNPs are rare, but most SNPs are biallelic (Doveri et al., 2008). This disadvantage of SNPs as compared to multiallelic SSRs markers is compensated to some extent by the relatively large abundance of SNPs. The abundance of SNPs in the genome provides a high density of markers near the interesting locus researcher. SNPs are evolutionarily stable markers and this is their advantage. They do not change from generation to generation and this low variability makes SNPs excellent markers for studying complex genetic features and a tool for understanding genomic evolution, as well as for identifying parasites (Rogers et al., 2012; Gujaria-Verma et al., 2016; Li X. et al., 2015). SNPs are also the dominant markers in biomedical applications due to the availability of data on the sequence of the human genome (due to what is known from the HapMap Project) and the knowledge of allelic variability (Altshuler et al., 2005).

The ability to screen a large number of individuals for SNP options allows predicting sensitivity to many diseases and creates conditions for personal medicine (Khatkar et al., 2007; Pavlovic S. et al., 2014). Thus, it is suggested that SNPs will still co-exist with other marker systems (Rafalski, 2002; Gupta et al., 2001). However, probably new technologies that reduce the cost of developing SNPs will find a wider application.

**CAPS (cleaved amplified polymorphic sequences).** They are also called as PCR-RFLP markers (due to being the combination of PCR and RFLP) (Maeda et al., 1990; Pouryasin et al., 2017; Bielikova et al., 2010). In this method, the DNA under study is amplified in PCR and then digested by restriction enzymes. If such changes as SNP and InDel occur in DNA, restriction enzyme recognition sites also change, resulting in DNA fragments having different lengths, thus polymorphism is clearly demonstrated.

The codominance and locus-specificity of CAPS-markers make it easy to distinguish homo- and heterozygous alleles. The need for a very low amount of DNA, the ability to differentiate codominant alleles, simplicity and cheapness - are the advantages of CAPS technology. With the use of CAPS markers, changes in the DNA sequence associated with the mutations create limitations and therefore the level of polymorphism is not as high as that of the SSR and AFLP markers. However, CAPS is widely used in mapping studies, on molecular tagging of genes (Filiz ve Koç, 2011). The primers used in this technology are developed based on the sequence information available in the genomics data banks or the cloned RAPD bands and DNA sequences. CAPS markers are universal and the possibility of finding polymorphism can be improved by combining CAPS with SCAR, AFLP, RAPD or SNPs markers (Agarwal et al., 2008; Shavrukov, 2016).

CAPS which are closely related to target genes are particularly useful in marker-assisted selection. Such CAPS markers are widely used in the selection of wheat, barley, soybean, potato, tomato and other plants to create disease-tolerant forms. Like many others, CAPS markers are also used to improve the plant's such important features as development, grain quality and cereal tolerance to pathogens, as well as the shape of tomato fruit (Shavrukov, 2016). CAPS markers are used in genotyping, in map-based cloning, and in gene identification studies (Spaniolas et al., 2006; Hou et al., 2010; Sandal et al., 2005)

**SCAR (sequence characterized amplified regions).** SCAR markers were first developed in 1993 for the genes for resistance to downy mildew of lettuce leaves (Paran and Michelmore, 1993). And later they started to be widely used for research of other plants (Busconi et al., 2006; Hernandez et al., 1999; Arnedo-Andres et al., 2002; Yuskianti and Shiraishi, 2010). Compared to RAPD markers, their reproducibility and specificity are higher. These markers are co-dominant and monospecific and they are mainly used for physical mapping. To develop SCAR primers, the procedures are performed as follows: after carrying out PCR, polymorphic bands are selected, the DNA sequence of the selected bands is sequenced. Sequence analysis of these polymorphic DNAs is performed through comparison with known DNA sequences present in the NCBI database (National Center for Biotechnology Information). This nucleotide sequence of polymorphic DNA is then used to synthesize specific SCAR primers.

**ISTR (Inverse sequence-tagged repeat).** ISTR markers are referred to as Retrotransposon-based molecular markers, like IRAP (Inter-Retrotransposon Amplified Polymorphism), REMAP (Retrotransposon-Microsatellite Amplified Polymorphism), iPBS (Inter Primer Binding Site amplification), S-SAP (Sequence-Specific Amplification Polymorphism), RBIP (Retrotransposon Based Insertion Polymorphism), ISAP (Inter Sine Amplified Polymorphism), etc.

Retrotransposons, especially LTR retrotransposons, form a significant part of the plant genome. Using the RNA mediator (intermediate) they are inserted into different parts of the genome

and cause mutations. Like all transposing elements they are present in the DNA of various parts of the chromosomes (in the telomeric, pericentromeric, centromeric parts, in introns and exons, in regulatory regions). Their number positively correlates with the size of the genome. For example, in the genome of A.thaliana they are much less than in the genome of Hordeum vulgare (Schulman and Kalendar, 2005). These properties and dynamism of LTR retrotransposons in plant genomes have made them excellent sources of molecular markers (Kalendar and Schulman, 2006; Gozukirmizi et al., 2015). The ISTR method is a multi-locus marker system based on the selective PCR amplification. The first ISTR primers were designed from coconut copia-like retrotransposon (Rhode, 1996). ISTR analyses of various plant species with the identical primers developed from coconut sequences have been discovered that these primers are universally applicable (Aga and Bryngelsson 2006).

**Sequence-based markers.** Molecular markers, which are based on identifying the sequence of individual DNA regions in a common pool of unknown DNA, are called Sequence-based markers. The development of this technology is due to the fact that markers based on hybridization are less reliable and less polymorphic. The advent of sequencing technologies such as next-generation sequencing (NGS) and genotyping by sequencing (GBS) caused dramatic changes in plant breeding by developing SNP, leading to high polymorphism (Davey et al., 2011, Jiang et al., 2016). Sequence-based markers are also used to study the evolution of primates (Bergey et al., 2013)

Due to high productivity, sequencing of the complete genome is achieved for economically important species such as rice, corn, soybean, sorghum, tomatoes, potatoes and Chinese cabbage, etc. While it is still difficult to use NGS technologies to mate the entire complex genome of plants such as barley and wheat. Within NGS technologies there have been identified thousands of SNPs that can be used to develop molecular markers in species with a complex genome. In addition, NGS is directly used in the detection of SNP, and several dozen genotypes can be progressively sequenced to collect ultra-dense genetic maps. In addition, various strategies are used to create partial genomes that can be used to directly monitor SNP using NGS technologies.

**EST technology.** For expression, each gene must be converted (transcribed) into mRNA. The mRNA formed during transcription serves as a template for protein synthesis during translation. However, there is a particular problem: mRNA is very unstable outside the cell. Therefore, a so-called enzyme, reverse transcriptase, is used to convert mRNA into cDNA (i.e., a reverse transcription process). cDNA which is much more stable than mRNA is an experimental DNA sequence because it is formed from mRNA. All mRNA in some tissue is a transcript that serves as a template for the synthesis of the cDNA library. These cDNAs refer to genes that are expressed in this tissue. Short segments (<100 bases) at one or both ends are sequenced on these cDNAs. These sequences - tags - carry within themselves sufficient information to identify the expression gene - they are called ESTs (Saccone and Pesole, 2003).

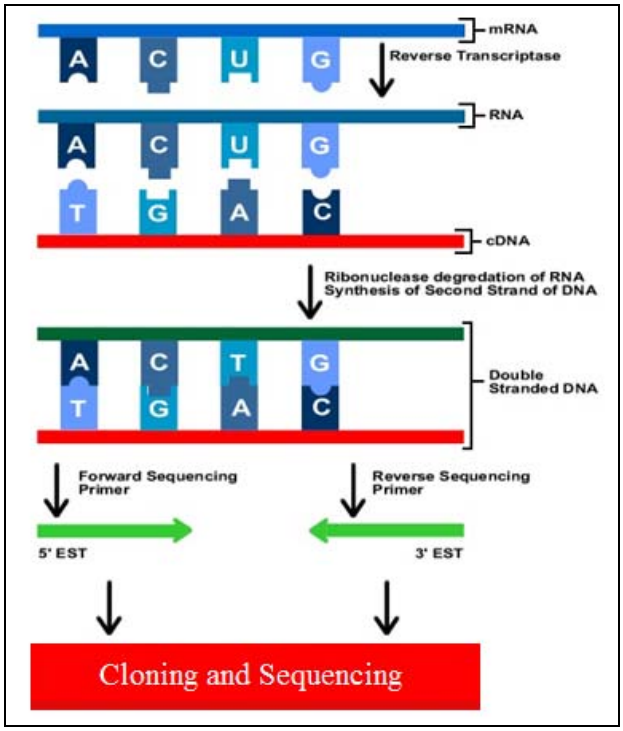


**Fig. 1.** Scheme of obtaining EST-tags of an expression gene.

EST sequences are used as a tool for gene identification, gene discovery, sequencing and the development of EST-based important molecular marker systems such as RFLP, SSR, SNP, CAPS. In addition, EST sequences can be used as probes for the determination of gene expression, in DNA microarrays, genetic linkage maps and physical maps (Davis et al., 1999). ESTs are a useful source for SSR markers, in various plant species the total share of SSR of 20 bp or more is 1 to 5% (Kantety et al., 2002).

SSR-regions are found in transcriptional loci, which are more conservative (which is the reason for their low polymorphism) than non-transcribed regions and their transfer to close species seems logical. Using primers obtained from EST sequences, amplification and sequencing of the relevant region can reveal many SNPs. More than

45 million ESTs have been created from 1,400 different species of eukaryotes. ESTs are used in areas such as phylogenetics, transcript profiling (or so called "expression profiling" is a quantitative study of the expression of the gene of many genes at the transcriptional level - at the RNA level) (Varshney et al., 2007).

**Interdisciplinary approaches in the development of molecular markers.** In recent years, new interdisciplinary approaches have been used to develop molecular markers. The mechanisms of response to radiation are conservative in plants and animals. The mechanisms of response to DNA damage (DDR) are the prevailing molecular mechanisms that are activated by radiation in plants and animals. This conservative nature of DDR in plants and animals can contribute to interdisciplinary researches which cross the traditional boundaries between plant biology and animal biology, which can expand the collection of markers currently used in REM (radiation exposure monitoring) for environmental and biomedical purposes.

Genes involved in trans-kingdom conservatively stored DDR networks often work with IR and UV-irradiation, deposited in biological databases. The authors adopted an innovative approach which uses data from available banks related to plants and humans to develop a "plant radiation dosimeter", i.e. based on DDR and plant genes, a platform that could serve as a marker for detecting the DNA damage and for assessing radiation-related risks for both environment and health (Nikitaki et al., 2017).

Quantification of DNA damage can be carried out on the basis of the analytical method of substances that induce DNA damage (LLC 4/16 2024APrinted in USA in 2016. Patents: www.moleculardevices.com/productpatents). This method is based on immunofluorescence detection of phosphorylated histone H2AX and 53BP1 protein. Therefore, the histone H2AX phosphorylation on serine 139 as well as the tumor suppressor protein 53BP1 can serve as a kind of marker for spontaneous or induced DNA damage. These molecular markers can be used to identify and characterize the mechanisms of action of genotoxicity of these agents. Since, with double-stranded DNA breaks, rapid phosphorylation occurs, this change can be used as an early and sensitive marker of double-stranded DNA breaks. In turn, 53BP1 protein is associated with repair processes, its phosphorylation and the formation of nuclear foci in response to DNA damage, as well as phosphorylation of H2AX, is an indirect but very sensitive method for determining double-stranded DNA breaks.

Using the specific - UV-B induced molecular markers, it is possible to study the mechanisms of the reaction of organisms to the impact. Some authors suggest that plants have at least 2 signal transduction mechanisms that regulate gene expression after UV-B absorption (Kalbina et al., 2008). Based on this approach, the nature of the dependence of fluence-response for mRNA transcripts of 4 molecular markers induced in A.thaliana by UV-B at 4 wavelengths in the range of 280-360 nm is found:

1) CHS (encoding chalcone synthase)

2) PDX1.3 (the coding enzyme involved in the formation of pyridoxine)

3) MEB5.2 (encoding a protein with unknown function, but very strongly regulated by UV-B)

4) LHCB1*3 (encoding xl a/b binding protein)

After analyzing the results, the authors concluded that there are 2 different UV-B responses: a) sensitive to rays in the range 300-310 nm: molecular markers CHS and PDX1.3 are regulated by a chromophore absorbing at a wavelength of 300 nm; b) sensitive to rays in the range 280-290 nm: molecular markers MEB5.2 and LHCB1* 3 are regulated by a chromophore absorbing at a wavelength of 280-290 nm.

Consequently, unlike many stress factors, the effect of UV radiation is highly specific and responses also differ in specific mechanisms and markers, what should be taken into account in the relevant studies.

## CONCLUSION

The development of new molecular methods of research expands the possibilities of the human mind in understanding the finest regulatory mechanisms present in the functioning of the genome of all living organisms. The knowledge gained thereby allows us to manage these mechanisms, to introduce useful ones into genomes, where they were previously absent (both hybridologically and genetically engineered). Therefore, molecular-marker technologies are considered as one of the most promising approaches in genetic analysis, in selection, in diagnostics, etc.

A multidisciplinary, inter-kingdom approach in the development of molecular markers is promising, as it promotes the creation of universal markers, which is economically most effective.

The existing close relationship between sequencing and molecular marker technologies promotes a more complete detection of single nucleotide polymorphism in the population, which is especially important in the personalization of

medicine.

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## Molekulyar Marker Texnologiyası: Müasir Vəziyyət Və Perspektiv İstiqamətlər (Icmal)

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Məqalədə elmin müxtəlif fundamental və tətbiqi sahələrində geniş tətbiq olunan molekulyar marker texnologiyaları haqqında müfəssəl informasiya təqdim edilmişdir. Məqalədə həmçinin mövcud molekulyar marker texnologiyalarının əksəriyyətinin xarakterik xüsusiyyətləri, onların üstün və çatışmayan cəhətləri göstərilmişdir. Genomun bir hissəsi olmaqla, molekulyar markerlər onun filogenez və ontogenezin gedişində baş verən dəyişiklikləri haqqında böyük informasiyanı özlərində daşıyırlar. Bu məqam molekulyar markerləri təkamülün, sistematikanın, adaptasiyanın genetik və epigenetik mexanizmlərinin öyrənilməsində, stres amillərinə qarşı yüksək davamlı formaların yaradılmasında, genetik xəritələrin yaradılmasında, genlərin identifikasiyasında, "genomun korreksiyasında" (genome editing), ətraf mühitin keyfiyyətinin müəyyənləşdirilməsində, eko – və genotoksikoloji problemlərin həllində, diaqnostikada, tibbi-məhkəmə ekspertizası praktikasında və s. sahələrdə mükəmməl alətə çevirir. Məqalədə həmçinin Hibridizasiya – və PCR - əsaslı molekulyar markerlərin yuxarıda xatırladılan müxtəlif məqsədlərlə tətbiq olunduğu çoxsaylı tədqiqatların nəticələri müzakirə edilir.

***Açar sözlər:*** *Molekulyar marker, restriksiya fraqmenti, polimorfizm, amplifikasiya, cpSSRs, ESTs*

# Молекулярно-Маркерные Технологии: Современное Состояние И Перспективные Направления (Обзор)

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В статье представлена подробная информация о молекулярно-маркерных технологиях, широко применяемых в фундаментальных и прикладных областях науки. Также изложены характерные особенности большинства существующих молекулярно-маркерных технологий, показаны их преимущества и недостатки. Будучи частью генома, молекулярные маркеры несут в себе огромную информацию об изменениях, происходящих в ходе онтогенеза и филогенеза. Это обстоятельство превращает молекулярные маркеры в совершеннейший инструмент в изучении эволюции, систематики, генетических и эпигенетических механизмов адаптации, в создании генотипов живых организмов, обладающих высокой устойчивостью к действию стрессовых факторов, в составлении генетических карт, идентификации генов, “коррекции генома” (genome editing), определении качества окружающей среды, диагностике, практике судебно-медицинской экспертизы, решении проблем эко - и генотоксикологии и др. В статье также обсуждаются результаты многочисленных исследований, в которых в вышеупомянутых целях были применены молекулярные маркеры, основанные на гибридизации и ПЦР.

***Ключевые слова:*** *молекулярный маркер, фрагмент рестрикции, полиморфизм, амплификация, cpSSRs, ESTs*