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# Применимость маркеров ISAP, ISSR и SSR в селекционных программах томата

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**РЕЗЮМЕ**

**За время тысячелетней селекции культурные растения характеризуются зауженностью генетической основы, отражающейся в одном и нескольких эффектах "бутылочного горлышка". В результате направленной селекционной работы потенциал имеющихся генетических ресурсов становится ограниченным, и требуется дальнейшая работа по поиску генресурсов для улучшения урожайности, устойчивости, пищевой ценности и т.д. С открытием современных методов генетики и биотехнологии некоторые достижения уже используются для улучшения потенциального использование генетических ресурсов. Среди этих методов индуцированный мутагенез можно рассматривать как наиболее полезный для традиционной селекции, хотя его широкое использование требует хороших знаний в области современных молекулярных технологий. В данной публикации мы сделали обзор по использованию SSR, ISSR и ISAP методов и привели примеры их конкретного применения в селекции томата.**

**Ключевые слова: томат, маркер-ассоциированная селекция, индуцированный мутагенез, микросателлиты, ISAP.**

# Applicability of ISAP, ISSR and SSR markers in tomato breeding programs

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**ABSTRACT**

**Domesticated crops are characterized by narrow genetic base reflecting one or more bottlenecks during millennia-long selection. As a result, current breeding programs are limited in available germplasm and are forced to deal with incremental improvements of yield, resistance, nutritional value, etc. Since the establishment of modern genetics and biotechnology, several new approaches have emerged to extend the genetic base and germplasm improvement. Among these methods, induced mutagenesis appeared as most useful conventional breeding tool. Although, its successful application currently requires good knowledge of modern molecular tools. In this paper we will make an attempt to overview SSR, ISSR and ISAP techniques as well as to offer examples of their application in tomato breeding programs.**

**Keywords: tomato, marker-assisted selection, induced mutagenesis, microsatellites, ISAP.**

## Introduction

Domesticated crops are characterized by narrow genetic base reflecting one or more bottlenecks during millennia-long selection process. Also, breeding process, especially early in history, can lead to further reduction of genetic diversity by excluding "inappropriate" plant material. As a result, current breeding programs are limited in available germplasm and are forced to deal with incremental improvements of yield, resistance, nutritional value etc. Since the establishment of modern genetics and biotechnology, several new approaches emerged to extend the genetic base and germplasm improvement.

These novel approaches and methods could be separated in several categories.

On first place there are achievements in plant genetics, biochemistry, physiology and molecular biology. They have laid the path to develop first *in vitro* techniques and, subsequently, allowed for modern plant biotechnology.

Second group comprises of *in vitro* techniques for propagation of plant cells and tissue as well as for plant regeneration. Its rise was based on achievements in plant biochemistry and physiology during the first half of XX century.

Third group comprises of molecular methods for detailed analysis and characterization of plant material. These methods were developed since 1970-ies but were vastly improved after the introduction of PCR in 1990-ies. Next, at the turn of the century, came the integration of molecular techniques with bioinformatics, which led to the quality leap of 'omics' technologies.

Despite the enormous repertoire of analytical and modifying methods, the main obstacle in plant breeding still lies in the limited genetic diversity of available germplasm. Breeders could rely on very few approaches. Historically, spontaneous mutations are among the first available sources. Another approach was the implementation of wide crosses and interspecies crosses. This approach offers excellent possibilities but was initially limited due to low survival rate of the hybrids. This was nearly overcame by embryo rescue method; still, the technique is laborious, time-consuming and sometimes non reproducible. Similar to this approach is the protoplast fusion. Unfortunately, this technique requires specialized equipment and highly trained personnel and, thus, is available to few laboratories.

Since 1980-ies another approach kicks in the mainstream – genetic engineering. Initially regarded as the ultimate tool in plant breeding, currently its practical application is unacceptable to the public and get limited to research only. Even recent developments in genome editing and gene control through RNA interference are treated as GM techniques and are banned from commercial applications in EU.

Fortunately, breeders can rely on another tool to improve genetic diversity – the induced mutagenesis (IM). In classic breeding programs induced mutagenesis was usually employed as treatment of the seeds with mutagenic factors (irradiation or chemical compounds) followed by phenotypic selection of required traits in the field. While being sufficient at the beginning, nowadays its successful application requires good knowledge of modern molecular analysis tools. As a result, induced mutagenesis is just one (but key one) step in an integrated breeding program.

The most critical issue in IM is how to identify the useful mutation. In theory, the ultimate approach is to sequence the entire genome of all mutant lines, to identify all changes in the sequence and to predict their effect on the phenotype. In a decade or two this might become an option, but today it is out of the reach of the scientific community. How can this problem be solved? Obviously, by implementing the most appropriate available analytical methods.

First, one should take care of traits sought after while bearing in mind that IM is a random process. As a consequence, upon treatment the mutant material will contain numerous DNA changes of all known types – point mutations, deletions, insertions, translocations, inversions, duplications, etc. Part of these changes will be incompatible with cell survival and they will be not present in the first progeny. Actually, this is the very first selection step in the process. The second step is the growth of the mutant lines with retained fertility in mind. If lines are infertile, a possible solution is to employ embryo rescue method if applicable. Usually at this stage only phenotype analysis is performed.

For breeding purposes, IM is often combined with backcrossing with line of interest. At this stage the key issue is to identify the mutant trait and to design a system for its tracking during backcross procedure – a marker. It is a complex task that requires sufficient theoretical background and experimental skills. The marker design could be split in three tasks – trait identification, choice of the analytical system and design of specific components.

**Trait identification approaches.**

For tomato, most valuable traits could be divided into several groups. First group consists of organoleptic-related traits like fruit color, shape and taste. Work on these traits do need specialized equipment or advanced methods since they are generated by complex biochemical and molecular genetic processes. Second group includes nutritional value traits – bioactive components, vitamins, microelements, etc. Evaluation of these traits generally is performed by chromatography methods. Third group consists of traits, related to resistance/tolerance to biotic or abiotic factors, while the fourth group of traits is relevant to the yield and market properties. Last two groups cannot be analyzed by visual means but need a complex experimental approach. Top of the list of available methods is occupied by the molecular biology techniques.

**Tomato breeding.**

Most domesticated crops are characterized with narrowed genetic base as compared to their wild relatives [1]. Tomato is no exception; its domestication involves two steps – one in Ecuador/Northern Peru and second one in Mesoamerica. Genetic analysis revealed that main bottleneck occurred during the transition between these two domestication centers. Exact nature of the events that led to the bottleneck is not known. Fortunately, the genetic base had expanded during last century due to successful introgression of new alleles from wild relatives [2, 3]. Nevertheless, the observed current phenotypic diversity is determined by still low genomic variability [4].

Current developments in tomato breeding rely not only on classic genetics but also on an array of molecular tools [5, 6, 7].

***Applications of SSR and ISSR markers in tomato breeding.***

Since its introduction in 1990-ies, microsatellite analysis became an important breeding tool along with RFLP, RAPD, AFLP [8] and SNP markers [9]. Development of microsatellite-based genomic map [10, 11, 12] paved the way to marker-assisted selection in tomato [13, 14, 15]. SSR employs naturally occurring variations of the number of repeats in the microsatellite motif (i.e. $CA_8$ vs $CA_9$) at a particular locus. Not all microsatellite loci of a given motif demonstrate variation of repeat numbers. The origins of such variations are not clearly understood as well as why some motifs are invariant while other demonstrate hyper variability. The main drawback of SSR is the need of preliminary sequence characterization of every microsatellite locus including neighbor sequences in order to design locus-specific primers. The main advantages of SSR are high reproducibility, high specificity of amplification as well as easy data incorporation for automatized genetic analyses. These features had determined the wide area of SSR applications.

Briefly, microsatellites were used to analyze a wide array of traits like genetic diversity [16, 17, 18], organoleptic properties [19], delayed fruit ripening [20] to name a few.

One trait of particular interest is related to carotenoid content [21, 22], which is directly related to organoleptic and marketing properties [23]. Marker-assisted selection was successfully performed to develop lines with increased beta-carotene concentration [24].

Microsatellite analysis was also implemented during development of "bio fortified" tomato lines [25] as well as for mass production of miraculin [26].

Microsatellite markers were also successfully applied for QTL pyramiding aimed to improving fruit quality [27].

A variant of microsatellite assay – ISSR, was used for characterization of genetic diversity [28]. ISSR exploits and detects naturally occurring variations in the length of the region between two microsatellite motifs, usually of same type. Experimentally, it resembles RAPD and can be performed using single primer corresponding to a particular microsatellite motif. The main advantages are better reproducibility than RAPD as well as technical simplicity. ISSR can be used for fast preliminary characterization of breeding material as well as for source of locus-specific molecular markers.

***Applications of SINE markers in tomato breeding.***

Another recently developed approach for marker-assisted selection is based on mobile genetic elements (MGE) in plant genome – ISAP method. The approach exploits two main characteristics – high number of MGE and their wide spread throughout genome. ISAP

method involves primers, specific for particular MGE and amplifies linker sequence. Thus, it incorporates high amplification specificity (hence high reproducibility) and good informativeness of the obtained amplification patterns. The high diversity of MGE in plants also offers a possibility do design different experimental schemes. Experimentally, ISAP technic is based on PCR, and it does not differ from standard SSR or ISSR methods, which makes it within the reach source for genome profiling.

The mapping efficiency was compared with microsatellite and AFLP methods and demonstrated similar potential [29]. Currently, a variant exploiting widespread SINE elements as anchors was designed – the ISAP [30]. The method was initially developed on potato but proved to work in pepper and tomato (with some limitations) (Tomlekova and Pantchev, unpublished results). Applicability of the original ISAP primer set was due to ortologous segments between the genomes. On the other hand, not all primers had generated amplification products, which reflect individual features of the tomato and pepper genomes (Nasya Tomlekova, unpublished results) and limits the successful application of ISAP in tomato breeding.

***Conclusion.***

SSR is the "golden standard" in plant molecular breeding due to its robustness, reproducibility and ease of data analysis with genetic programs. In tomato genetic assays, SSR can generate patterns with Polymorphic Information Content (PIC) value ranging from 0.22 to 0.82 [31]. The only major drawback is the need for preliminary identification and characterization of variable microsatellite loci.

ISSR is appropriate for rapid initial screening for polymorphic loci in the breeding material even with low genetic variability, resulting in PIC value of 0.29 [32]. Also, ISSR can be the method-of-choice for preliminary characterization of local tomato populations.

ISAP is a relatively new method that can complement SSR. In tomato, obtained PIC value ranges are comparable to that of SSR (Nasya Tomlekova, unpublished results). Unfortunately, despite the potential, ISAP needs further adaptation to tomato-specific SINE sequences. This is the main obstacle to wider implementation of this robust technique in tomato breeding programs.

Marker-assisted selection became an important tool in tomato breeding. Hence, its successful application relies on extensive scientific knowledge on tomato biology [33]. Among the numerous methods and technologies, microsatellite analysis is one of the most used throughout the world due to relative simplicity and high reproducibility [34, 35, 36]. Despite the current achievements, new marker technologies like ISAP are needed to meet the future challenges in food demand [37].

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