

**THE USAGE OF MUTANT GENOTYPES IN TOMATO SELECTION FOR
SPECIFIC TRAITS AT THE INSTITUTE FOR VEGETABLE CROPS**

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In order to find a superdominant gene for yield, a collection of 350 samples with genes mutations and different expression in different phases of growth and development: fruit size, leaf shape, plant architecture, number of fruits, etc., has been studied (through multi-year research). Current seed production at the Institute for Vegetable Crops in Smederevska Palanka includes 26 hybrids and two in acceptance process, of different purposes and characteristics. The most common mutation genes in tomato selection at the Institute are: *sp* (*self-pruning*) inbuilt in determinant hybrids (Balkan F1, Marko F1, Rebus F1) and varieties (Narvik SPF, SP-109, Adonis), *u* (*uniform ripening*) - Lido F1, Atina F1, Danubius

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F1, *rin* (ripening inhibitor)- Nada F1, Šampion F1, Sef F1, Sidra F1, Rebus F1, *j* (jointless) – Narvik, Adonis, SP-109, *c* (potato leaf) - Mi-13, Enigma F1, Rebus F1, *Ph -2* (*Phytophthora infestans* resistance) – Luna F1, Zlatni jubilej F1, Sef F1. Quality of tomato fruits is defined by two groups of genes which determinate the organoleptic traits and nutrition composition. Organoleptic quality includes taste and smell, colour and texture of fruits established in variety differences, nutritive regime of plants, maturity phase and yield. Little is known about gene regulation, responsible for variation of these traits.

Key words: mutant genotypes, specific traits, tomato breeding

Genetic resources

Tomato belongs to *Solanaceae* family together with 2800 other species and some of them are economically significant (potato, pepper and eggplant), ornamental species (*Petunia* spp. and *Nicotiana* spp.) and medicinal species (*Datura* spp. *Capsicum* spp. and *Nicotiana* spp.). All tomato species originate from South America, Ecuador and Chile and they are grown even in some variable and extreme habitat. Genetic resources of traditional tomato include nine wild and related varieties FOOLAD (2007), and the collection is publically available (TGRC, <http://tgrc.ucdavis.edu/indek.cfm>). Even though the introduction of tomato narrowed the genetic basis, it is still possible to explore the variability of germplasm, especially towards specific aims in breeding programmes. For some traits it is necessary to use wild species in order to complete the gene found for better yield in optimal conditions and in stress conditions (BARONE *et al.*, 2009). Genetic variability is available from the wild germplasm, which usually has negative effect, but the challenge is to identify and use only favourable alleles. Wild germplasm used in traditional tomato breeding programs through F₁ interspecies hybrids, whose progeny is used for segregation for molecular and conventional breeding is *S. lycopersicum*. By using molecular markers, some of the progeny is also used for obtaining new genetic resources such as constant near-isogenic populations (NIL) and introgression lines (IL). There are IL populations deriving from tomato wild species such as: *S. pennellii* *S. habrochaites* *S. pimpinellifolium* *S. lycopersicoides* *S. chmielevskii* *S. sitiens* (FERNIE *et al.*, 2006) and consists of the large number of homozygote lines, all with mark for defining segments from the wild genome. Lately, the collection T-DNK insertion mutagenesis has been made in different tomato varieties (LOZANO *et al.*, 2008). TILLING mutant populations are also in development in few countries and could be the additional source of genetic variability.

Table 1. Common and specific aims of tomato breeding, different in the way of use (fresh or proceeded) (Modified FOOLAD, 2007)

Universal aims	Aims in selecting tomato for fresh consumption	Aims in selecting tomato for fresh proceeding
High yield	Round and compact fruit	Determinant growth of plants
Resistance to diseases	Firmness and shelf life of fruits	Concentrated flowering
Wide adaptability	Uniform fruit size	Mechanized yield
Early ripening	Shape and colour of fruit	Easy separation from the petiole
Adjusting the ripening during optimal temperatures	The look of fruit	Colour of fruit
Resistance to cracking caused by irrigation	Absence of stains on the fruit	pH of fruit and total acid
Tolerance to fruit rot	Fruit texture	Soluble substances
Well foliated plants	Taste and smell of the fruit	Viscosity

Tomato is a vegetable crop very rich in number and types of molecular markers and their location is being mapped. At the moment, more than 2500 markers, including RFLP, EST, SSR and COS, are mapped on 12 tomato chromosomes (FRARY *et al.*, 2005). Mapping of genes for resistance to viruses, bacteria, nematodes and fungi has become priority in breeding tomato. Major genes, that controlled disease resistance are intensively researched and mapped.

Using the absence of abscission on floral stem in selecting tomato varieties for industrial proceeding

One of the specific traits of tomato varieties for proceeding and mechanized yield that has intensively been improved is the absence of peduncle abscission layer. Trait that hardens the mechanized yield is when a part of the petiole is separated together with the fruit. Part of the peduncle that stayed on the fruit mechanically damages the fruit during transport and directly decreases the fruit quality and the quality of pulp during the mashing. Great importance for growing tomato for mechanized yield is the presence of *jointless* gene which impacts the absence of pedicel on floral branch and the zone of separation of the fruit from the peduncle is between the fruit and the very petiole.

Trait *jointless* is caused by three recessive genes: *j-1*, *j-2* i *j-2ⁱⁿ* (*jointless-2 incomplete*). Trait *jointless-1* is not acceptable for practical breeding work since the great foliation of plants cannot be overcome and the yield is drastically decreased. In

practical selection the *j-2* is much more useful since it causes the absence of pedicel and *j-2ⁱⁿ* which forms the rudimental peduncle abscission layer which does not have the role of abscission, and which is more important fruits can be separated from the petiole without peduncle remaining. The nature of *jointless* genes, its pleiotropic effects and linkage with other genes has been explained by RICK and SAWANT (1956 and 1960) such as: *sp* (*self-pruning*), *mc* (*macrocalyx*), *pi* (*pistilate*), *bu* (*buchy*), *bg* (*blue green*), *wt* (*wilty*), *ici* (*inclinata*) and *ps* (*positional sterile*). Mutant *j-2* (*jointless-2*) has been discovered by RICK (1956) in wild form of tomato *Lycopersicon pimpnelifolium*. Allele tests proved that genes are located in different locus. Furthermore, RICK (1960) found its linkage with genes for elongated peduncle, complex peduncle, hypertrophic calyx and elongated fruit, which made it acceptable in breeding programs. ROBINSON (1980) implies that *j-2* has negative pleiotropic effects which harden the breeding of tomato: great number of flowers per peduncle which causes the appearance of flowered plants, slow ripening of fruits and appearance of deformed fruits. This problem in the practical breeding has been overcome by using epistatic gene effect *o* (*ovata*) – gene for elongated shape of the fruit. The third gene *j-2ⁱⁿ* has been found by JOUBERT (1962) and it is on a special locus. Expressions of this gene is similar to expression of the wild form (presence of abscission zone), but abscission zone is absent and morphologically it is hard to define the presence of gene *j-2ⁱⁿ*. morphologically it can be determined only when fruit ripening starts.

MARKOVIC (1984) determined the relations in mutual crossing of all three genes bearers of peduncle abscission layer absence on the petiole of the fruit and wild type. These researches represent the beginning of the breeding program at the Institute for Vegetable Crops. Recombined lines and breeding lines introduced from France (INRA) were the start material for this breeding program. The result of this program were varieties SP-109 (*j-2*) introduced on variety list in 1980, Narvik SPF (*j-2ⁱⁿ*) introduced on variety list in 1987 and a little bit later variety Adonis (*j-2ⁱⁿ*) introduced on variety list in 2000.

Recently, anatomic specifics of abscission zone which represents the hydraulic barrier for the transport of the water to the fruit are being researched. Abscission zone could be the key for tomato resistance to drought so these three genes could be researched once again regarding water transport and the possibility of reduced watering followed by minimal yield reduce (MILOSEVIC *et al.*, 2005).

Selection for the better quality of tomato fruits

Quality of tomato fruits includes several aspects that can be grouped in two categories: organoleptic traits and content of nutrient. Organoleptic quality implies taste and smell but also colour and texture of fruits and the influence of variety differences, nutrition regime, maturity phase during the yield, shelf life. General relations have been determined between chemical and physical characteristics of tomato fruits (ORDONEZ-SANTOS *et al.*, 2008) or sensor traits (CAUSSE 2003), but little is known about the genetic control and genes responsible for their variability. It is necessary to know genetic control and the external influence on regulating tomato metabolites that cause variations in fruit quality. This is mostly due to tomato

compounds such as carotene (mostly lycopene and alpha carotene, precursor of vitamin A), ascorbic acid-vitamin C, and phenol compounds (flavonoids and derivatives of hydroxycinnamic acid).

At the Institute for Vegetable Crops a lot has been done both on organoleptic traits and on the content of nutritive matters. Breeding work is based on programme that accumulates a large number of genes for enlarging the number of lycopene. Lycopene is unique in tomato fruit and represents highly valued antioxidant. Morphologically lycopene gives a red colour to the tomato fruits. Line selection in the past 15 years resulted in decreased level of lycopene (ZDRAVKOVIĆ *et al.*, 2002, ZDRAVKOVIĆ *et al.*, 2003b). Decreasing the level of lycopene led to parallel breeding program for fruit firmness, which included inbuilt of inhibitor gene of maturity, mostly *rin* (*ripening inhibitor*). Inbuilt of this gene almost all parameters of bioactive matters effecting the quality have been decreased up to 50%, especially of lycopene, alpha carotene, vitamin C, flavonoids, total sugars and soluble substances (CVIKIĆ *et al.*, 2000, 2003, MARKOVIĆ *et al.*, 2010). The main sources of lycopene have been used from the local populations and old, domesticated varieties among them some genotypes with extremely high values up to 80 mg/% - (most common value is 25-40 mg/%) (MARKOVIĆ *et al.*, 2000, MARKOVIĆ *et al.*, 2007).

The level of lycopene is genetically caused trait, but it is under a strong external influence. The level of lycopene can be changed due to decreased or increased potassium through fertilization (ZDRAVKOVIĆ *et al.*, 2004b, ZDRAVKOVIĆ *et al.*, 2007). Agriculture aspect of irrigation and reduced irrigation treatments in circumstances of an acceptable yield, significantly alter or increase the dry matter content in the fruit and thus content of lycopene - which increases (SAVIĆ *et al.*, 2004).

High level of bioactive matters besides lycopene, such as vitamin C, alpha carotene, precursor vitamin A, phenol compound put tomato into highly nutritional food and at the same time very popular in organic farming (ZDRAVKOVIĆ *et al.*, 2010). There are great demands for breeding tomato for specific uses and ways of production that is not recognized lately, but this program started at the beginning of this century (MARKOVIĆ *et al.*, 1999).

Heredity of bioactive matters depends on large number of genes and each component is inherited separately. Polygene heredity of these parameters is not clear enough so the selection at the Institute for Vegetable Crops is guided by accumulation of each parameter separately (BARONE *et al.*, 2009). Breeding is time consuming and moves are relatively small but all attention has been given to increasing the quality, especially since the selection for fruit firmness jeopardizes the identity since it decreases the level of bioactive matters that define this vegetable crop. Tomato has been put in medicinal plants lately due to its specificity. Commercial hybrids selected at the Institute for Vegetable Crops with high level of bioactive matters are: Danubius F1 (introduced at the variety list in 1997) and Atina F₁ (1998), Zlatni jubilej F₁ (2000). All these hybrids contain gene *u* (*uniform ripening*), which means they possess the possibility of full ripening and their bioactive content of lycopene and other matters has not been destroyed (MARKOVIĆ

et al., 2000, ZDRAVKOVIC *et al.*, 2002, ZDRAVKOVIC *et al.*, 2003b, MARKOVIC *et al.*, 2007).

Fruit firmness

Fruit firmness gives good sensor traits to tomato fruits. Genotypes with firm fruits are suitable for transport and have long shelf life. Selection program to fruit firmness belongs to specific goals in breeding tomato for fresh consumption (FOOLAD, 2007). This program gave a large number of hybrids for this purpose (MARKOVIC *et al.*, 2008) Institute for Vegetable Crops has five hybrids with inbuilt fruit firmness genes: Nada F₁ (2004), Sampion F₁ (2006), Sef F₁ (2009), Sidra F₁ (2010), Rebus F₁ (2010). All these hybrids have ripening inhibitors: *rin-ripening inhibitor*, *nor-non ripening*, *Nr-never ripe* in their pedigree.

Fruit firmness can be accomplished by stopping the maturation process, since fruits do not overripe and get soft. Changed aspect of ripening influences the quality of fruits regarding bioactive substances (ZDRAVKOVIC *et al.*, 2003a, ZDRAVKOVIC *et al.*, 2004c).

In order to avoid the loss of bioactive matters, parental lines of commercial hybrids were lines with high values of these matters, or as for hybrid Marko F₁ (introduced on list in 2002), by accumulating firmness of separate selection of each component: fruit firmness, firmness fruit epidermis and pericarp thickness (MARKOVIC *et al.*, 2008, ZDRAVKOVIC *et al.*, 2008).

The nature of *rin* gene, most commonly used in commercial hybrids for increasing the fruit firmness is recessive single gene. In homozygote condition, fruits do not ripe, totally block synthesis of ethylene and characteristic red colour does not appear. Seed in the fruits ripens in 110-120 days from germination, which is usual for fruits without this mutant (ZDRAVKOVIC *et al.*, 2004c). When gene is in heterozygote condition characteristic colour appears in progeny (mature fruits) and fruit is capable to last longer and to keep the satisfactory quality (ZDRAVKOVIC *et al.*, 2009). This trait is called "shelf life" and all hybrids with this ripening inhibitors in their pedigree have long shelf life. This trait is used when fruits are shelved for long period so bringing the ethylene in the storage causes the ripening (ZDRAVKOVIC *et al.*, 2004b).

Resistance to diseases

Up to now, more than 40 great resistance genes and many QTL have been localized on tomato gene map. Such information is used for more efficient selection with molecular markers (marker-assisted selection, MAS). Presently MAS is being used on tomato for various traits. This includes selection for vertical resistance to viruses such as *Tobacco mosaic virus* and tomato spotted virus, to nematodes and fungi, such as *Fusarium* and *Verticillium*, *Phytophthora* and other (FRARY *et al.*, 2005). MAS is also used for pyramiding if more than one gene determinates resistance of variety or breeding line (BARONE *et al.*, 2005).

Late blight is one of the most common diseases of tomato, it occurs almost every year and makes great economical losses. Control of this pathogen with

fungicides is not always sufficient. Solution to this problem is in growing less sensitive or more resistant cultivars of tomato. Resistance of tomato to *P. infestans* is a rather complicated problem due to a great variability of inheritance of tomato and physiological cultivars (MIJATOVIC *et al.*, 2007). Hybridization is being done between tomato genotypes carrier of *Ph-2* genes of resistance to this parasite and tomato genotypes with good production traits for yield and fruit quality but sensitive to this parasite. Selected tomato lines and hybrids showed higher level of resistance comparing to sensitive parents (MIJATOVIC *et al.*, 2007, ZDRAVKOVIC *et al.*, 2004a). First resistant genotypes to some *Phytophthora infestans* isolates have been created in 1950 and in 1960. This resistance has been controlled by *Ph -1* gene. However, during the strongest epidemics of tomato late blight these genotypes were sensitive. Significantly higher resistance to this parasite showed genotypes with inbuilt *Ph -2* gene 1968. The *Ph-2* gene was used by LATERROT (1975) to breed Peraline. This gene has been incorporated in several commercial hybrids Luna F₁, Zlatni jubilej F₁. Lately, hybrid Sef F₁ has been created which is a bearer of both genes of resistance to tomato late blight. Researchers detected 2 genes of resistance to causer of late blight: *Ph-3* and *Ph-5* (FOOLAD *et al.*, 2008). There are no clean lines for using these genes in applicable selection.

Besides tomato late blight very significant diseases are caused by: *Verticillium daliae* and *Fusarium oxysporum* f. sp. *lycopersici* (MIJATOVIC *et al.*, 2008). High tolerance to verticillium wilt caused by *Verticillium daliae* has been inbuilt in large number of hybrids: Balkan F₁, Luna F₁, Sampion F₁, Sef F₁. In order to obtain certain resistance to causer of tomato fusarium wilt *Fusarium oxysporum* f. sp. *lycopersici*, three genes have been used in breeding commercial hybrids (DJORDJEVIC *et al.*, 2010).

The authors of this paper are the group of leading tomato breeders at the Institute for Vegetable Crops. Živoslav Markovic, PhD wrote a part of this paper related to the research in the field of the monogenic inheritance of certain tomato traits. Gene resistance to some tomato diseases were studied for more than three decades under the leadership of phytopathologist Mirjana Mijatovic, PhD. The use of individual genes and their incorporation in experimental and commercial hybrids at the Institute for Vegetable Crops, has been the subject of the study for researchers Jasmina Zdravkovic, PhD and Milan Zdravkovic, PhD, in terms of yield, plant architecture, earliness, fruit quality, resistance to biotic and abiotic environmental factors. By using biotechnological methods the researcher Nenad Pavlovic, PhD opens a new chapter in the selection and use of genetic polymorphism of tomato for processing purposes, with special attention to food safety and nutritional value.

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KORIŠĆENJE MUTIRANIH GENOTIPOVA U SELEKCIJI PARADAJZA NA SPECIFIČNA SVOJSTVA U INSTITUTU ZA POVRTARSTVO

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Da bi se našao superdominantan gen za prinos paradajza, ispitana je kolekcija od 350 uzoraka (kroz višegodišnja ispitivanja), sa mutacijama gena koji imaju različitu ekspresiju u različitim fazama rasta i razvića paradajza: veličinu ploda, oblik listova, arhitekturu biljke, broj plodova, itd. Sadašnja semenska proizvodnja Instituta za povrtarstvo obuhvata 26 hibrida i dva hibrida u procesu priznavanja, različitih namena i karakteristika. Najčešće korišćeni mutirani geni korišćeni pri selekciji paradajza u Institutu za povrtarstvo su: *sp* (*self pruning*) koji je ugrađen u determinantne hibride (Balkan F1, Marko F1, Rebus F1) i sorte (Narvik SPF, SP-109, Adonis), *u* (*iniform ripening*) - Lido F1, Atina F1, Danubius F1, *rin* (*ripening inhibitor*) - Nada F1, Šampion F1, Sef F1, Sidra F1, Rebus F1, *j* (*jointless*) – Narvik, Adonis, SP-109, *c* (*potato leaf*) - Mi-13, Enigma F1, Rebus F1, *Ph -2* (*Phytophthora infestans resistance*) – Luna F1, Zlatni jubilej F1, Sef F1. Kvalitet plodova paradajza definišu dve grupe gena koji određuju organoleptička svojstva i hranljivi sastav. Organoleptički kvalitet podrazumeva ukus i miris, boju i teksturu plodova koje su uslovljene sortnim razlikama, nutritivnim režimom biljaka, fazom zrenja i berbom. Malo se zna o genetskoj regulaciji koja je odgovorna za varijaciju ovih osobina.

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