GENOTYPICAL VARIABILITY IN PROGENY OF TRANSGENIC INTERSPECIFIC HYBRID OF TOMATO. COMMUNICATION I. ISOLATION OF SELECTIVE MATERIAL

Yu.V. Chesnokov¹, N.I. Bocharnikova², L.V. Esaulova¹

¹ N.I. Vavilov All-Russia Research and Development Institute of Plant Growing, RAAS, St.Petersburg 190000, Russia
e-mail: ychaschenkov@vir.nw.ru

² All-Russia Research and Development Institute of Vegetable Crop Selection and Seed-Growing, RAAS, Moscow province, Odintsovo region, VNISOK, settlement 143080, Russia
e-mail: gamtas@mail.ru

All-Russia Research and Development Institute of Vegetable Crop Selection and Seed-Growing, RAAS, St.Petersburg 190000, Russia
e-mail: lesaaulova@mail.ru

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Summary
The authors made the phenotypic, genetic-selective and molecular analysis of genotypes of progeny of transgenic interspecific hybrid of tomato of Lycopersicon esculentum Mill. (the Fakel variety) × Solanum pennellii Cor. The high genetic variability was revealed in studied variants both on molecular and phenotypic level. Selected tomato forms are of interest for practical breeding.

Keywords: interspecific hybrid of tomato, coadapted blocks of genes, spectrum genotypic variability, alien exogenous DNA.

Creation of cultivated plant species with improved adaptability necessitates the transfer into their genomes of wild species’ chromosome loci or groups of coadapted genes for economically valuable quantitative traits (1). However, many genes in these blocks are linked with other genes providing undesirable properties. Potential genotypic variation in the progeny of interspecific hybrids directly depends on meiotic recombination peculiarities and the interaction of genetic factors associated with distinctions between components of a cross. Consequently, a practical use of the wild species’ gene pool requires effective methods for increasing the available range of genetic and genotypic variability, which is one of the most important tasks of contemporary selection. Today, the use of exogenous DNA is assumed to have significant prospects owing to the targeted transfer of determinants from donor to recipient and increased variability in a progeny.

Previously, the authors have obtained the transgenic interspecific hybrid of tomato Lycopersicon esculentum Mill. (Cv. Fakel) × Solanum pennellii Cor. (2). Its paternal form was the species-source of valuable adaptive traits—cold hardness and resistance to a number of viral and bacterial diseases (3). A distinctive feature of this hybrid is its genotype carrying foreign DNA for resistance to kanamycin introduced into the germinating pollen during a natural pollination—fertilization (2).

The purpose of this work was to estimate breeding, genetic and molecular features of genotypes in a splitting population of the transgenic interspecific hybrid of tomato carrying foreign exogenous DNA.

Technique. The object of study were transgenic plants the interspecific hybrid of tomato L. esculentum Mill. (Cv. Fakel) × S. pennellii Cor. carrying the determinant for resistance to kanamycin (2). Phenotypic characteristics of hybrid plants and their progeny were assessed in field conditions by comparing with known standard genotypes of tomato (3, 4).

Total DNA was extracted from fresh leaves using the method proposed by D.B. Dorokhov and Je. Klocke (5). PCR-analysis was performed in the reaction mixture (25 ul volume) containing 1 × buffer from a commercial kit (“Biomaster”, Russia), 2 mM MgCl₂, 100 uM of each dATP, dCTP, dGTP and dTTP, 2,5 pmol/ul of the appropriate primer, 0,25 units Taq-polymerase and 20 ng genomic DNA. RAPD-analysis was performed using the oligonucleotide primers OPA-09 (GGGTAAAGCC) and OPA-01 (CAGGCCCTTTC) (“Operon Technologies”, USA), primers 2-70-1 (GTCTCGTCCG), 4-70-1 (GCCCTCTTGT), 3-70-1 (TCCCTGTGCCC), 2-60-1 (GAGTCTGTCCG) (“Genosys”, USA) and the primer P-01 (AGCAGCGTCC) designed in the Center "Biotechnology" (RAS, Moscow).

Polymerase chain reaction was carried out in the thermocycler AM-PLY-250 (“Biokom—Service”, Russia) under the following regime: 3 min at 94 °C, 1,5 min at 37 °C, 1,5 min at 72 °C – 1 cycle; 1 min at 94 °C, 1,5 min at 37 °C, 1,5 min at 72 °C – 33 cycles; 1 min at 94 °C, 1,5 min at 37 °C, 15 min at 72 °C – 1 cycle. Amplification products were separated by electrophoresis in 2% agarose gel in 0,5×TBE buffer, stained with ethidium bromide and photographed.

Statistical processing of data was performed according to B.A. Dospekhov (6).

Results. Nine transgenic genotypes of F₁ L. esculentum × S. pennellii resistant to kanamycin had been selected in preliminary studies (2, 7). A control – interspecific hybrids obtained without the use of exogenous DNA. Already in F₁, hybrid transgenic plants manifested intrapopulation diversity by fruit coloration, fruit weight and number of seeds per fruit, which traits were distinct from control as well. Fruit color varied from orange-red to yellow and yellow-green. Among them, two F₁ genotypes contrasting in color and weight of fruits were selected for a further work. In following F₂ generations, populations were split in shape and size of plant, sterility, fertility, color and weight of fruits (Fig. 1, A).

The studied populations demonstrated common inherent traits along with particular features of individual transgenic genotypes. Thus, fruit color in the entire control population was yellow-green, while transgenic F₁ genotypes developed red, orange-red, orange, yellow-orange, yellow and yellow-green fruits. The two selected F₁ genotypes - 2-83 (2) and 2-83 (4) had, respectively, red and yellow fruits. In four generations of descendants resulting self-pollination of these plants, fruit color remained unchanged (red and yellow), but reciprocal crosses of genotypes 2-83 (2) and 2-83 (4) provided the F₂ with uniform red color of fruits.

In the red-fruited population 010 (F₂ of hybrid 2-83 (2)), it was detected the genotype 010/37 with a very complex inflorescence cluster including up to 300 flowers. Though, self-pollination of these plants gave the next generation having the cluster "scattered" into smaller groups of flowers (up to 50-60 pcs). It’s really interesting to study the nature of inheritance of this trait. Instability
of this feature was also proved by further studies of following splitting generations. Though, such complex clusters were partially sterile (heterostyly of flowers and partially sterile pollen), which complicated the analysis.

In the same population (010), self-pollination of the large-red-fruited genotype 010/1 resulted in F2 progeny splitting by the ratio 3:1 (χ² = 2.84; 0.05 < P < 0.20); about ¼ plants developed normal clusters with fruits, ¼ - inflorescences with reduced or modified sterile flowers. Self-pollination of the descendants with normal flowers resulted in F3 splitting as 1/3 (not splitting, normal fertile inflorescences) and 2/3 (plants with normal and reduced / modified inflorescences). In this F3 population, the number of forms with normal and modified / reduced inflorescences was close to the ratio 3:1 (χ² = 1.93; 0.05 < P < 0.20), which feature was stably inherited over the next generations (F4 and F5). The authors separated these genotypes to create the lines with a modified and reduced cluster. Both traits were found to be monogenic recessives. Homozygous (recessive) forms of these genotypes are sterile, so maintenance and propagation of these mutant forms is possible only via the heterozygote. The newly found trait "modified cluster" has some morphological resemblance to the known mutation fa (8).

In the yellow-fruited population 011 (F2 of the hybrid 2-83(4)), there were detected genotypes 011(9) and 011(17) whose seedlings manifested the character close to the known mutation c ("potato" leaf) (Fig. 1, B) (8). In F2 progeny of the genotype 011(9), the number of such plants was minor (5 of 82) and statistically insignificant. A similar trend was also observed in further generations (F3 and F4): "potato leaf" was inherited randomly, not being subject to statistical criteria and rules.

The genotype 011(17) manifested a quite different nature of inheritance of this character. Like 011(9), F2 population obtained by self-pollination of 011(17) were the seedlings splitting by normal and "potato" leaves as approximately 3:1 (χ² = 1.23; 0.20 < P < 0.50). These two types of plants were self-pollinated. "Potato leaf" F2 individuals resulted in F3 with only this trait, while F2 forms with normal leaves gave a diverse F3: 1/3 plants with no split (only normal leaf type) and 2/3 splitting by leaf type (normal and "potato leaf" as 3:1). F4 the self-pollination progeny of hybrid 011(17) manifested the similar tendency as in F2 and F3. Thus, the trait "potato leaf" has variable nature of inheritance: in the genotype 011(17) - stable, recessive, monohybrid, homozygous, in 011(9) - random and unstable. These facts can be concluded as different nature of “potato leaf” phenotype in these samples.

Morphological analysis of following generations (F3-F4) revealed a high phenotypic heterogeneity of populations including new types of plants not peculiar to both parents. Thus, in F5 of the splitting population 011, it was detected the genotype 2-82(6) with strong pubescence of seedlings (Fig. 1, B) resembling to the known expression of dominant mutations Wo or Ln (woolly and lanata – respectively, thick and excessive pubescence). This trait remained during the growing season along with other features. Adult plants of the sample 2-82(6) exhibited long creeping stem (a wild-type trait), complex inflorescences (up to 30-40 flowers) and red fruits weighted 50-60 g (cultivar traits). Self-pollination of these plants resulted in F4 and subsequent generations (up to F8) showing this trait unchanged, as well as the lines obtained upon them.

At the same time, F2-F8 manifested changes in a number of other characteristics. In F4, a compact bush type emerged (cultivar trait) and the yellow-orange and violet color of fruits, while fruit weight remained almost unchanged. The split by inflorescence type was observed as well as the first type - cluster of 30-40 flowers, the second type - only 8-10. The compact bush type was observed in F2 as well, and following generations didn’t split by stem length. The long-stem F4 provided F5 splitting into pubescent and normal plants. In F5-F8 of highly pubescent plants, this trait was conserved. Plants with a normal bush type gave in F8 no individuals with thick pubescence, while in F3 this trait emerged and was inherited by F8. Fruit color was inherited in F3-F8 independent on other traits and unchanged in both red-fruited and yellow-orange-fruited genotypes.

Inflorescence type was characterized by extreme phenotypic diversity. In both samples (with clusters of 30-40 and 8-10 flowers) self-pollination in F2-F8 provided quite variable descendants with simple (4-5 flowers) and complex cluster (up to 40 flowers or more). Even within a family, this trait was unstable and unpredictable.
Qualitative characteristics of a flower (structure and morphology) varied too. In particular, self-pollination contributed to F\textsubscript{5} with emergence of genotypes with reduced cluster, in F\textsubscript{4} and F\textsubscript{5} — forms with a leathery type of flower (all parts including sepals have dense epidermis). Such flowers had undeveloped stamens and were sterile.

Sterility of different degrees was also observed in other genotypes of this family. In almost all generations of highly pubescent plants (F\textsubscript{2}-F\textsubscript{5}), fertility (development of fruits) varied from 0 to 100%.

There were established no correlations between thick pubescence, bush type, inflorescence type, sterility and fertility of flowers. The nature of inheritance wasn’t revealed as well: statistical analysis of data showed no trends and similarities to Mendelian laws or any other laws of biological inheritance.

It should be noted that the genotype F\textsubscript{1} originally carried the active transgenic DNA sequence, which was established by dot-blot-hybridization with subsequent determination of NPTII activity (neomycin-phosphotransferase that determines resistance to kanamycin as selective marker) (2). The comparative RAPD analysis of studied populations showed in progeny of transgenic hybrid the dominance of RAPD-spectra similar to those of \textit{L. esculentum}, and only in some genotypes — segments of amplified DNA of \textit{S. pennellii}, which confirmed their hybrid nature. A number of genotypes showed the absence of certain DNA areas typical to \textit{L. esculentum} and new DNA regions uncharacteristic for parental forms (Fig. 2). In many hybrids, new zones of amplicons indicating significant modifications of the genome were found.

Thus, the use of exogenous DNA in interspecific crosses contributes to the wide range of genotypic variability in tomato and the rapid creation of hybrid genotypes with economically valuable features, in contrast to conventional hybridological methods spending years to develop a similar result. (9-12). The obtained genetic material is of great interest for genetics and breeding work, because these populations include the plants with conventional recombinant traits, new transgressive features and unusual mutant types not peculiar to parental forms.

REFERENCES