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(54) Title: TOMATO PLANTS HAVING FRUIT WITH YELLOW AND RED SEGMENTS

(57) Abstract: The present invention relates to tomato plants having fruit with red and yellow segments that appear across the fruit from the internal seed area to the most external layer of the epidermis. The present invention discloses that this phenotype, designated *Arlecchino*, is linked to insertion mutation within the *Phytoene synthase 1 (Psy1)* gene.

TOMATO PLANTS HAVING FRUIT WITH YELLOW AND RED SEGMENTS

FIELD OF THE INVENTION

The present invention relates to tomato plants having fruit with bicolor red-yellow flesh and epidermis, comprising within their genome an insertion mutation within the *Phytoene synthase 1* (*Psy1*) gene.

BACKGROUND OF THE INVENTION

Tomato is a basic nutritional component in many cultures worldwide. It is known for its vitamin, mineral and anti-oxidant content that provide the consumers with these health beneficial components. A large number of tomato varieties is available in the market, and it is recognized that in addition to the nutritional and taste parameters, color and general appearance influence the purchase of tomato fruit by private as well as cuisine professional customers.

Tomato fruit color is furnished by lycopene, a linear carotene molecule with 11 conjugated double bonds, which accumulates to high concentration within chromoplasts during fruit ripening. Biosynthesis of lycopene is mainly controlled at the gene expression level of carotenoid-biosynthesis enzymes. During fruit ripening, genes for enzymes upstream to lycopene are upregulated whereas genes for lycopene cyclases, which metabolize lycopene to alpha- and beta-carotene, are silenced.

The improvement of crop species has been a fundamental human pursuit since the beginning of agriculture. One can imagine that unique plant phenotypes, sometimes represented even by a single plant, caught the eyes of the first ancient breeders. The manner in which the improvement process was done constituted a founder effect which is often manifested in severe genetic bottlenecks. As a result of these genetic bottlenecks imposed during early domestication and modern breeding activities, cultivated varieties of most crop species carry only a small fraction of the variation presented in their wild ancestors and land races (Tanksley S D and McCouch S R. 1997. Science 277:1063–1066). The narrowed genetic variation in modern crops is one of the causes for genetic vulnerability to various plant diseases and pests. Moreover, it presents a breeding obstacle by lowering the chance of obtaining better gene and allelic

combinations to improve traits with agricultural importance.

One of the most powerful and efficient method in the area of genetics, molecular biology and plant breeding is based on mutant variation. Mutation is the major evolutionary force that creates variation to improve survivability of existing diversity and for the evolution of novel ecotypes, races and species. However, since the frequency of spontaneous mutants is very low, they supply information on a relatively small number of genes and biological phenomena. Therefore, artificial mutagenesis methods have been developed and are being applied to induce variation. Induced variation is in turn used as a tool for the discovery of gene function and for understanding developmental processes.

An available source of tomato mutations is an isogenic tomato "mutation library" generated in the genetic background of the processing tomato inbred variety M82, by one of the inventors of the present invention and co-workers (Menda N et al., 2004. Plant J. 38:861-872). For generating the library, a total of 13,000 M2 families, derived from ethyl methanesulfonate (EMS) chemical treatment and from fast-neutron mutagenesis of seeds, were phenotyped when grown under field conditions. Based on the phenotypes, the families were categorized into a morphological catalog that included 15 primary and 48 secondary categories. More than 3000 mutations have been identified in this library. Some of the mutations represent new alleles of previously described phenotypes from the monogenic mutant collection of The Tomato Genetics Resource Center (TGRC), most of which in the M82 isogenic population. In addition, over 1,000 novel phenotypes with multiple alleles per locus were identified.

Results of allelism tests between mutants sharing similar phenotypes verified the hypothesis that the population is reaching saturation, with hits in the majority of the genes (for example, from the dominant *LANCEOLATE* mutation 8 independent alleles where identified; from the yellow fruited *Yellow flesh* mutation 5 alleles were obtained). In addition, screening of 1,000 EMS families subjected to two treatments of EMS did not result in novel phenotypes.

The potential of wild species as a source of genetic variation to bring about crop improvement was recognized early in the twenty first century (Zamir D. 2001. Nat Rev Genet. 2:983–989). Initial interspecific breeding attempts met with severe problems,

including incompatibility in crosses between the wild species and the cultivated crops; F1-hybrid sterility; infertility of the segregating generations; reduced recombination between the chromosomes of the wild species and the cultivated crop; and tight linkage between genes that have negative effects and the traits of interest (McCouch S. 2004. PLoS Biol. 2). Despite these obstacles, there are many examples in which wild introgression breeding has made a considerable contribution to the development of modern-day varieties, predominantly as a source for monogenic or sometimes called single gene traits and to a smaller extent for complex traits such as yield, compositional quality and resistance to various stresses that are influenced by quantitative trait loci (QTL; Fernie A R et al., 2006. Curr. Opin. Plant Biol. 9:196–202).

Notwithstanding the above-described tools for generating new tomato phenotypes, there is a constant market demand for stable cultivars having fruit with new, attractive color.

SUMMARY OF THE INVENTION

The present invention relates to tomato cultivars producing fruit with yellow-red segments across the entire fruit, from the internal placenta and/or locules and to the external epidermis layer of the pericarp. This yellow-red stripe phenotype, designated herein *Arlecchino,* can include from one stripe or segment of each color in a single fruit to multiple number of stripes of each color in the fruit. In contrast to hitherto known fruit with alternate yellow-red skin phenotype, the *Arlecchino* phenotype shows the color section across the entire fruit.

The present invention further relates to seeds of the plants of the present invention, to plants grown from the seeds, to their progeny, to fruit produced by the plants, to plant parts derived therefrom and to methods of producing same.

The present invention is based in part on the unexpected discovery of a mutation in a population of tomato backcross inbred lines originated from a man-made cross of wild species tomato with a commercial cultivar. The mutation, an insertion mutation within the *Phytoene synthase 1* (*Psy1*) gene is linked to the *Arlecchino* phenotype described above. The yellow sections of the *Arlecchino* phenotypes result from an insertion within the *Psy1* gene, in intron 8 present between exon 8 and exon 9 of the

gene (according to the gene structure as depicted in Figure 8, an update of the structure published by Giorio G et al., 2008. FEBS J. 275:527-535).

The initial insertion observed in yellow sections of the *Arlecchino* fruit was of nine (9) nucleotide, comprising the nucleic acids sequence ATCTGGATA (SEQ ID NO:1). The position of this insertion within the *Psy1* gene indicated a direct repeat of eight (8) nucleotides comprising the nucleic acids sequence TCTGGATA (SEQ ID NO:2) separated by one Adenine (A) nucleotide. Further analysis of the insertion using high-fidelity DNA Polymerase (PrimeSTAR GXL DNA Polymerase (Takara Bio)), cloning and sequencing the amplified polynucleotides revealed an insertion of a transposon flanked by the nucleic acids sequence set forth in SEQ ID NO:2 associated with the *Arlecchino* phenotype. Nevertheless, a PCR product comprising the nucleic acid sequence TCTGGATAATCTGGATA (SEQ ID NO:7), comprising the direct repeat separate by the Adenine (A) nucleotide is amplified from genetic material obtained from yellow sections of *Arlecchino* fruit using standard DNA Polymerase (READY-MIX kit (Syntezza).

In cells of the yellow sections the insertion is found in homozygous form. The red sections of the *Arlecchino* phenotype comprise either wild type *Psy1* alleles or *Psy1* alleles comprising transposon excision footprint comprising variable sequences of the direct repeat area within the intron.

Without wishing to be bound by any specific theory or mechanism of action, this hitherto unknown mutation may be the result of merging divergent genomes achieved through man-made genetic crossings.

According to one aspect, the present invention provides a tomato cultivar which produces fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to at least one allele of r^{arl}, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

According to certain embodiments, cells of the yellow segments of the *Arlecchino* fruit are homozygous for the r^{arl} allele.

According to certain embodiments, the insertion comprises a transposon flanked

by the nucleic acid sequence TCTGGATA (SEQ ID NO:2) at the transposon 3'end. According to additional embodiments, the insertion comprises a transposon flanked by the nucleic acid sequence ATCTGGATA (SEQ ID NO:1) at the transposon 3'end.

According to certain embodiments, the transposon belongs to the hAT family. According to some embodiments, the transposon comprises a nucleic acid sequence at least 90% or at least 95% or more homologous to the nucleic acid sequence set forth in SEQ ID NO:3. Each possibility represents a separate embodiment of the present invention. According to certain exemplary embodiments, the transposon comprises the nucleic acid sequence set forth in SEQ ID NO:3. According to additional exemplary embodiments, the transposon consists of the nucleic acid sequence set forth in SEQ ID NO:3.

According to certain embodiments, the insertion within the *Psy1* gene comprises a nucleic acid sequence at least 90%, at least 95% or more homologous to the nucleic acid sequence set forth in SEQ ID NO:4. According to certain exemplary embodiments, the insertion within the *Psy1* gene comprises the nucleic acid sequence set forth in SEQ ID NO:4. According to additional exemplary embodiments, the insertion within the *Psy1* gene consists of the nucleic acid sequence set forth in SEQ ID NO:4.

According to certain exemplary embodiments, the r^{arl} allele of *Psy1* comprises the nucleic acid sequence set forth in SEQ ID NO:5.

According to certain embodiments, the wild type (wt) *Psy1* allele comprises the nucleic acid sequence set forth in SEQ ID NO:6.

According to some embodiments, the tomato cultivar comprises at least one pericarp cell homozygous for the r^{arl} allele and at least one pericarp cell comprising at least one wild type *Psy1* allele or at least one *Psy1* allele comprising transposon excision footprint.

According to certain embodiments, the transposon excision footprint comprises at least one nucleotide deletion within the nucleic acid sequence TCTGGATAATCTGGATA (SEQ ID NO:7). According to some embodiments, the transposon excision footprint comprises at least two, at least three, at least four, at least 5, at least 6, at least 7 or at least 8 nucleotides deletion. Each possibility represents a separate embodiment of the present invention

According to some embodiments, the fruit is ripening fruit at the breaker stage and onward. According to some exemplary embodiments, the fruit is a fully ripe fruit.

It is to be explicitly understood that the entire tomato fruit can show the yellow–red stripe or segment phenotype or the stripes/segments can appear only on parts of the fruit. The width of the stripes can also be variable such that a single fruit may comprise from one red segment and one yellow segment covering the entire fruit to a multiple number of narrow red and yellow stripes covering all or part of the fruit. All appearances are encompassed by the present invention.

According to some embodiment, the tomato cultivar produces small fruit (cherry-like fruit). According to certain exemplary embodiments, the tomato cultivar is *Solanum lycopersicum*.

According to additional embodiments, the tomato cultivar further comprises within its genome an additional *Psy1* mutant allele encoding for a *yellow flesh* phenotype. According to some embodiments, the *Psy1* gene encoding for the *yellow flesh* phenotype comprises the nucleic acid sequence set forth in any one of SEQ ID NO:8 and SEQ ID NO:9. According to these embodiments, the *Arlecchino* tomato cultivar comprises at least one r^{arl} allele, at least one *Psy1* mutant allele encoding for a *yellow flesh* phenotype and at least one wild type *Psy1* allele or *Psy1* allele comprising transposon excision footprint. According to certain exemplary embodiments, the yellow segments of the *Arlecchino* fruit comprise one r^{arl} allele and one *Psy1* mutant allele encoding for the *yellow flesh* phenotype and the red segments comprise wild type *Psy1* allele and/or *Psy1* allele comprising transposon excision footprint.

According to yet additional embodiments, the tomato cultivar is suitable for commercial growth. The tomato cultivars advantageously can further comprise beneficial agronomical traits as are well known in the art including, but not limited to, high germination rate, herbicide resistance, insect resistance, resistance to bacterial, fungal or viral diseases, resistance to various types of non-biotic stress, male sterility, vigorous growth and any combination thereof. These traits may form part of the genetic background of the tomato cultivars or may be introduced by any method as is known to a person skilled in the art, including, but not limited to, breeding, single trait conversion and transformation.

According to another aspect, the present invention provides a tomato cultivar homozygous to the r^{arl} allele, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion of a transposon flanked by the nucleic acid sequence TCTGGATA (SEQ ID NO:2) at the transposon 3' end, wherein the tomato cultivar produces entirely yellow fruit. According to certain exemplary embodiments, the tomato cultivar producing the entirely yellow fruit is homozygous to the r^{arl} allele comprising the nucleic acid sequence set forth in SEQ ID NO:5.

The present invention also provides seeds of the tomato cultivar of the invention wherein plants grown from the seed produce fruit having an *Arlecchino* phenotype of yellow-red segments spanning from placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to r^{arl} allele, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

Pollen and ovules from the tomato cultivars of the present invention; the seeds produced from same and the plants grown from the seeds and fruit produced by these plants and having the *Arlecchino* phenotype are also encompassed within the scope of the present invention.

A tissue culture of regenerable cells or parts thereof of the tomato cultivar of the invention, the regenerable cells obtained from a plant part selected from the group consisting of leaves, pollen, embryos, roots, root tips, anthers, flowers, fruit and seeds, is also encompassed within the scope of the present invention, as well as plant regenerated from the tissue culture producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to r^{arl} allele, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

The tomato cultivar plants of the present invention can be in the form of stable true-breeding lines or as a more diverse material, all of which comprise within their genome the r^{arl} allele.

According to additional aspect, the present invention provides a tomato fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta

and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to r^{arl} allele in a cultivated tomato plant producing the fruit, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

According to certain exemplary embodiments, the fruit having the *Arlecchino* phenotype comprises at least one cell homozygous for the r^{arl} allele. According to additional exemplary embodiments, the r^{arl} allele comprises the nucleic acid sequence set forth in SEQ ID NO:5.

According to another aspect, the present invention provides a method for producing a tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, the method comprising introducing into a tomato cultivar producing red fruit or a part thereof a genetic element comprising r^{arl} allele of *Phytoene synthase 1*, the r^{arl} allele comprises an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

According to certain embodiments, the insertion comprises a transposon flanked by the nucleic acid sequence TCTGGATA (SEQ ID NO:2) at the transposon 3'end. According to additional embodiments, the insertion comprises a transposon flanked by the nucleic acid sequence ATCTGGATA (SEQ ID NO:1) at the transposon 3'end.

According to these embodiments, the transposon comprises a nucleic acids sequence at least 95%, 96%, 97%, 98%, 99% or 100% homologous to SEQ ID NO:3. Each possibility represents a separate embodiment of the present invention.

According to certain exemplary embodiments, the *Psy1* r^{arl} allele comprises the nucleic acid sequence set forth in SEQ ID NO:5.

Any method as is known in the art can be used to introduce the genetic element comprising the r^{arl} allele into the tomato cultivar producing red fruit or to a part thereof. When the genetic element is introduced to a plant part, including, but not limited to, a seed, a cell or a tissue, the method further comprises regenerating a cultivar tomato plant from the seed, cell or tissue. Any method as is known in the art for regenerating a plant from seeds, cells or tissues can be used.

According to certain embodiments, the genetic element is introduced by crossing the tomato cultivar producing red fruit with a donor tomato plant comprising the genetic element to provide offspring cultivated tomato plants. According to these embodiments, the method further comprises the steps of:

a. examining a nucleic acid sample obtained from each offspring cultivated tomato plant or par thereof for the presence of r^{arl} allele;

b. selecting offspring cultivated tomato plants comprising the r^{arl} allele; and

c. examining the fruit produced by the plants selected in step (b) and electing cultivated tomato plants producing fruit with *Arlecchino* phenotype.

According to other mboidments, the genetic element is introduced by transforming a plurality of cells of the tomato cultivar producing red fruit with said genetic element. According to these embodiments, the method further comprises:

a. examining a nucleic acid sample obtained from each transformed cell for the presence of r^{arl} allele;

b. selecting a plurality of cells comprising the r^{arl} allele;

c. regenerating the plurality of transformed cells to obtain a plurality of transgenic plants comprising the r^{arl} allele; and

d. examining the fruit produced by the transgenic plant and selecting plant producing fruit having the *Arlecchino* phenotype.

According to certain embodiments, the method further comprises selfing, at least once, the selected cultivated tomato plant to produce a progeny and further identifying and selecting cultivated tomato plants comprising the r^{arl} allele and having the *Arlecchino* phenotype.

Any method as is known in the art for examining the nucleic acid sample for the presence of the r^{arl} allele can be used according to the teachings of the present invention.

According to some embodiments, the present invention provides at least one probe or pair of primers specifically detecting the presence of the r^{arl} allele of *Phytoene synthase 1*.

According to certain embodiments, the pair of primers is designed to amplify an

r^{arl} allele marker comprising the nucleic acid sequence set forth in SEQ ID NO:7 (TCTGGATAATCTGGATA). According to certain exemplary embodiments, the r^{arl} allele marker is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:10 (CAGTGCCAGAAGAGGAAGA) and SEQ ID NO:11 (TTGCGGTACAAGACCAAAGA).

According to additional embodiments, the pair of primers is designed to amplify the full length transposon insertion. According to certain exemplary embodiments, the transposon is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:12 (GTGGATCCTGAAATGGCTTG) and SEQ ID NO:13 (AGTACTAATAAAATGGTTTTGCC).

According to yet additional embodiments, the pair of primers is designed to amplify the 3' genomic junction of the transposon insertion within the *Psy1* allele. According to certain exemplary embodiments, the 3' transposon insertion junction is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:14 (GGGCTAGTCGGTGTATCAT) and SEQ ID NO:11 (TTGCGGTACAAGACCAAAGA).

According to yet further embodiments, the pair of primers is designed to amplify the 5' genomic junction of the transposon insertion within the *Psy1* allele. According to certain exemplary embodiments, the 5' transposon insertion junction is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:15 (CTGGAAGGGTGACCGATAAA) and SEQ ID NO:16 (ATGATACACCGACTAGCCC).

According to another aspect, the present invention provide a method for producing a tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, the method comprises mutating at least one allele of *Phytoene synthase 1* with an insertion mutation. According to certain embodiments, the insertion is within an intron resulting in a non-functional *Psy1* allele. According to additional exemplary embodiments, the insertion is within intron No. 8 located between exon 8 and exon 9 of the *Psy1* gene having the nucleic acid sequence set forth in SEQ ID NO:6, resulting in *Psy1* splice variants encoding non function protein.

According to yet additional aspect, the present invention provides an isolated polynucleotide encoding a mutated *Phytoene synthase 1* comprising the nucleic acids sequence as set forth in SEQ ID NO:5.

Other objects, features and advantages of the present invention will become clear from the following description and drawings.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows the construction of the *Solanum pimpinellifolium* (*Sp*) LA1589 backcross inbred line (BIL) population and the production of the *Arlecchino* mutation detected only in a single plant in the BIL#112 family in the year 2010.

FIG. 2 shows the *Arlecchino* mutation phenotype in different genetic background showing whole fruit (A) and transverse sections (B).

FIG. 3 demonstrates the segregation of fruit color traits in two independent F2 populations resulting from crosses of BIL#112 showing the *Arlecchino* phenotype and wild type red tomato lines.

FIG. 4 shows the carotenoid concentration (µg/g Fresh Weight) in fruit samples of red (r) and yellow (y) sectors of *Arlecchino* compared with wild type (M82) and the *yellow-flesh* mutant r3756.

FIG. 5 demonstrates the nucleotide sequence differences in the transposon insertion/excision site within the *Psy1* gene in red and yellow sections of *Arlecchino* fruit. Yellow sections were homozygous for the flanking repeats whereas red sections were heterozygous to this sequence because the transposon was excised from one allele.

FIG. 6 is a schematic demonstration of the amplified segments of *Psy1* transcripts obtained from different color sections of *Arlecchino* fruit.

FIG. 7 is a schematic demonstration of the transposon and flanked nucleic acid insertion within the *Psy1* gene (Fig. 7A) and of the amplified *Arlecchino* marker (Fig. 7B).

FIG. 8 shows the nucleic acids sequence of the r^{arl} allele (SEQ ID NO:5). Upper case letters indicate sequences of tomato wild type *Psy1* gene, with exons marked in bold

letters. Small case letters indicate the *Arlecchino* intron sequence and the flanked nucleotide insertion is boxed.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides tomato cultivars producing fruit having the appealing appearance of red and yellow stripes or segments that span across all the edible part of the fruit – from the inner seed area and up to the most external layer of the exocarp (fruit skin), through the mesocrap and endocarp. The present invention further discloses for the first time the genetic configuration which is linked to this appearance, designated herein *Arlecchino*.

Definitions

The term "plant" is used herein in its broadest sense. It also refers to a plurality of plant cells that are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a root, stem, shoot, leaf, flower, petal, fruit, etc. As used herein, the term "plant part" typically refers to a part of a tomato plant, including single cells and cell tissues such as plant cells that are intact in plants, cell clumps and tissue cultures from which tomato plants can be regenerated. Examples of plant parts include, but are not limited to, single cells and tissues from pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, and seeds; as well as pollen, ovules, leaves, embryos, roots, root tips, anthers, flowers, fruits, stems, shoots, scions, rootstocks, seeds, protoplasts, calli, and the like.

The term “pericarp” as is known in the art refers to the wall of a matured ovary. Specifically, tomato fruit pericarp refers to the fruit wall, which surrounds the seeds and placenta. The term “pericarp” includes the exocarp, mesocarp and endocarp as well as the radial pericarp.

The term "gene", as used herein, refers to a hereditary unit consisting of a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a particular characteristics or trait in an organism. The term "gene" thus refers to a nucleic acid (e.g., DNA or RNA) sequence that comprises coding sequences necessary for the production of RNA or a polypeptide or its precursor. A

functional polypeptide can be encoded by a full-length coding sequence or by any part thereof as long as the desired activity or functional properties (for example, enzymatic activity, ligand binding, signal transduction, etc.) of the polypeptide are retained. The term "parts thereof" when used in reference to a gene refers to fragments of that gene. The fragments may range in size from a few nucleotides to the entire gene sequence minus one nucleotide. Thus, "a nucleic acid sequence comprising at least a part of a gene" may comprise fragments of the gene or the entire gene. The term "gene" encompasses both cDNA and genomic forms of a gene.

The term "gene" also encompasses the coding regions of a structural gene and includes sequences located adjacent to the coding region on both the 5' and 3' ends for a distance of about 1 kb on either end such that the gene corresponds to the length of the full-length mRNA. The sequences which are located 5' of the coding region and which are present on the mRNA are referred to as 5' non-translated sequences. The sequences which are located 3' or downstream of the coding region and which are present on the mRNA are referred to as 3' non-translated sequences.

As sued herein, the term “allele” refers to alternative or a variant form of a gene or of any kind of identifiable genetic element, which are alternative in inheritance because they are situated at the same locus in homologous chromosomes. Such alternative or variant forms may be the result of single nucleotide polymorphisms, insertions, inversions, translocations or deletions, or the consequence of gene regulation caused by, for example, chemical or structural modification, transcription regulation or post-translational modification/regulation. In a diploid cell or organism, the two alleles of a given gene or genetic element typically occupy corresponding loci on a pair of homologous chromosomes.

As used herein, the terms “r^{arl} allele” or “r^{arl} allele of P*sy1*” are used herein interchangeably and refer to a *Phytoene synthase 1* allele comprising an insertion within intron of the allele, wherein the insertion results in a non-functional splice variant of Psy1. According to certain exemplary embodiments, the terms refer to the nucleic acid sequence set forth in SEQ ID NO:5.

The term “genotype” as used herein refers to the genetic constitution of a cell or organism. As is known in the art, a genotype can relate to a single locus or to multiple

loci, whether the loci are related or unrelated and/or are linked or unlinked. In some embodiments, an individual's genotype relates to one or more genes that are related in that the one or more of the genes are involved in the expression of a phenotype of interest (e.g. color trait as defined herein). Thus, in some embodiments a genotype comprises a summary of one or more alleles present within an individual at one or more genetic loci.

The term “phenotype” as used herein refers to the appearance or other detectable characteristic of an individual, in particular individual plant. According to certain embodiments, the phenotype results from the plant genotype. According to additional embodiments, the phenotype results from the interaction of its genome, proteome and/or metabolome with the environment.

The terms "segment", "section" and their plurality forms are used herein interchangeably and refer to area of the tomato fruit which is either yellow or red in color, forming the red-yellow phenotype of *Arlecchino*.

As used herein, the term "breeding", and grammatical variants thereof, refer to any process that generates a progeny individual. Breeding can be sexual or asexual, or any combination thereof. Exemplary non-limiting types of breeding include crossings, selfing, doubled haploid derivative generation, and combinations thereof.

As used herein the term “selfing” refers to a controlled self-pollination of a plant, i.e. contacting pollen and ovule produced by the same plant. The term “crossing” refers to controlled cross-pollination, i.e. contacting pollen and ovule each produced by a different plant.

The term "donor", as used herein, refers to the plant or plant line from which the trait, introgression or genomic segment originates, and which donor may have the trait, introgression or genomic segment either heterozygous or homozygous.

The term "recipient", as used herein, refers to the plant or plant line receiving the trait, introgression or genomic segment from a donor, and which recipient may or may not have the trait, introgression or genomic segment itself either heterozygous or homozygous.

The term “offspring” as used herein refers to any plant resulting as progeny from

a vegetative or sexual reproduction from one or more parent plants or descendants thereof. For instance an offspring plant can be obtained by cloning or selfing of a parent plant or by crossing two parent plants and include selfing as well as the F1 or F2 or still further generations. An F1 is a first-generation offspring produced from parents at least one of which is used for the first time as donor of a trait, while offspring of second generation (F2) or subsequent generations (F3, F4, and the like) are specimens produced from selfing of F1s, F2s and the like. An F1 can thus be (and in some embodiments is) a hybrid resulting from a cross between two true breeding parents (true-breeding is homozygous for a trait), while an F2 can be (and in some embodiments is) an offspring resulting from self-pollination of the F1 hybrids.

As used herein, the term "hybrid" refers to any offspring of a cross between two genetically unlike individuals, including but not limited to the cross between two inbred lines.

As used herein, the term "inbred" means a substantially homozygous individual plant or plant line.

As used herein, the term "backcross", and grammatical variants thereof, refers to a process in which a breeder crosses a hybrid progeny back to one of the parents, for example, a first generation hybrid F1 with one of the parental genotypes of the F1 hybrid. In some embodiments, a backcross is performed repeatedly, with a progeny individual of one backcross being itself backcrossed to the same parental genotype.

A "cultivated tomato plant" or “tomato cultivar” or “tomato cultivar plant” is understood within the scope of the invention to refer to a plant of the *Solanaceae* clade *Lycopersicon* that is no longer in the natural state but has been developed by human care and for human use and/or consumption. "Cultivated tomato plants" or “tomato cultivars” or tomato cultivar plants” are further understood to exclude those wild species which comprise the trait being subject of this invention as a natural trait and/or part of their natural genetics. Examples of tomatoes include *Solanum lycopersicum* (formally *Lycopersicon esculentum*), *Solanum cerasiforme*, *Solanum cheesmanii*, *Solanum chilens*e, *Solanum chmielewskii*, *Solanum hirsutum*, *Solanum parviflorum*, *Solanum pennellii*, *Solanum peruvianum*, or *Solanum lycopersicoides*. According to certain embodiments, the tomato cultivar is *Solanum lycopersicum* (taxonomy according to

Peralta I et al. 2005 Northern Peru Systematic Botany 30(2):424–434.

The term "heterozygous" is used herein to refer to unlike alleles at one or more corresponding loci on homologous chromosomes.

The term "homozygous" is used herein to refer to like alleles at one or more corresponding loci on homologous chromosomes.

New appearances of tomato fruit that would be appealing to the customer are always desired. The sophisticated customer also requires that the fruit are firm, tasty, and have reasonable shelf live. The tomato grower is looking for a plant that is resistant to biotic and abiotic stress and produces high yield. Understanding the genetic inheritance rules in plants and the fast development of molecular genetics tools during the past decades facilitates the production of superior agricultural crops in general and tomato plants in particular.

In the course of studying the phenotype-genotype relationship in a population of tomato backcross inbred lines (BILs) produced on a background of a population with artificially induced mutations the inventors have unexpectedly produced a tomato plant having fruit with alternate yellow and red segments. This phenotype has been designated as "*Arlecchino*". In contrast to hitherto known fruit showing similar external fruit phenotype, the colored segments of *Arlecchino* were not restricted to the epidermis and/or outer layers of the fruit pericarp, but spanned from within the fruit (including the placenta and/or locules) across the pericarp and up to the most external epidermis layer (Figure 2).

The color of the fruit as indicated herein refers to the color of ripening fruit at the breaker stage and up to a fully ripe or mature fruit. At the breaker stage there is a definite break of color from green to tannish-yellow, pink or red on the tomato fruit surface. The term "mature" as used herein means that the contents of two or more seed cavities have developed a jellylike consistency and the seeds are well developed. The *Arlecchino* phenotype is easily detected visually.

Tomato fruit is classified as a fleshy berry. As a true fruit, it develops from the ovary of the plant after fertilization. Tomato fruit can be either bilocular or multilocular. Most cultivated varieties except cherry tomatoes have two to five locules. The locules are surrounded by the pericarp. The pericarp includes the inner wall, columella; the

radial wall, septa; and the outer wall (epidermis). The pericarp and the placenta comprise the fleshy tissue of the tomato. The seeds are located inside of the locular cavities and are enclosed in gelatinous membranes. There are vascular bundles throughout the outer wall of the pericarp and travelling from the stem to the center of the tomato and from there radiating to each seed.

The BILs population from which the *Arlecchino* mutated phenotype was isolated was constructed from a cross between the small red-fruited, self-compatible, wild accession of *Solanum pimpinellifolium* LA1589 and the *S. lycopersicum* processing-tomato, inbred variety, cv. E6203 (TA209). Without wishing to be bound by any specific theory or mechanism of action, the *Arlecchino* mutation may be the results of a "genomic shock" resulting from wide crosses.

One of the factors characterizing mutagens is that changes in the DNA are created via a variety of molecular mechanisms and thus the repertoire of the mutations obtained can vary dramatically among different mutagens (transitions, transversions, deletions or additions). Another known force that induces mutations results from the creation of interspecific populations by the crossing of evolutionary divergent types. Merging of divergent genomes can create a "genomic shock", a process described by McClintock (1984. Science 226:792-801). Despite intensive research, the molecular mechanisms that affect a genomic shock are not well characterized. However, it has been reported that the introgression of alien genomic segment into a divergent background can trigger genetic changes and mutations. This phenomenon has been previously described, a detailed work executed in wheat being an example (Shaked et al., 2001. Plant Cell 13:1749-1759). Shaked et al. have shown that upon the synthesis of new wheat allotetraploids events such as gene loss, gene silencing and activation are rather common. In wheat, interspecific hybridization followed by chromosome doubling leads to rapid, genetic and epigenetic changes, where retrotransposons appear to be the principal actors when their activity is activated by the genomic shock (Shaked et al., 2001, *ibid*; Ozkan et al., 2001. Plant Cell 13:1735-1747; Kashkush et al., 2003. Nat. Genet. 33:102--106).

Further breeding of the isolated *Arlecchino*-phenotype plant provided for the tomato plants of the present invention, which are cultivar tomato plants suitable for

commercial growth.

The present invention further discloses the linkage between the *Arlecchino* phenotype and the presence of transposon insertion in at least one allele of the *Phytoene synthase 1* (*Psy1*) gene. It is to be explicitly understood that the presence of the r^{arl} allele is obligatory for the *Arlecchino* phenotype, but may not be the only factor responsible for its appearance.

Sequencing the area of the initially observed insertion of the 9 base pairs (SEQ ID NO:1) within the *Psy1* gene pointed to the presence of a direct repeat of the nucleic acid sequence TCTGGATA (SEQ ID NO:2). Insertion of transposons into a plant genome is typically characterized by the formation of sequence duplication in direct orientation at the place of insertion. As exemplified hereinbelow, the inventors of the present invention have discovered that the *Arlecchino* phenotype is indeed a result of transposon insertion in cells forming the fruit yellow sections. The insertion is within intron 8 of the *Psy1* gene (according to the *Psy1* sequence shown in Figure 8) resulting in splice variants missing the last exon (exon 9) of *Psy1* such that translation of a functional protein is impaired. The red sections cells comprise either wild type *Psy1* alleles and/or cells in which the transposon has been excised without negatively affecting the gene transcription, enabling the translation of functional *Phytoene synthase 1*.

The present invention thus provides a tomato cultivar which produces fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to at least one allele of r^{arl}, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

According to certain embodiments, cells of the yellow segments of the *Arlecchino* fruit are homozygous for the r^{arl} allele.

According to certain embodiments, the insertion comprises a transposon flanked by the nucleic acid sequence TCTGGATA (SEQ ID NO:2) at the transposon 3'end. According to additional embodiments, the insertion comprises a transposon flanked by the nucleic acid sequence ATCTGGATA (SEQ ID NO:1) at the transposon 3'end.

According to certain embodiments, the transposon belongs to the hAT family. According to some embodiments, the transposon comprises a nucleic acid sequence at least According to these embodiments, the transposon comprises a nucleic acids sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% homologous to SEQ ID NO:3. Each possibility represents a separate embodiment of the present invention.

According to additional exemplary embodiments, the transposon consists of the nucleic acid sequence set forth in SEQ ID NO:3.

According to certain exemplary embodiments, the insertion within the *Psy1* gene comprises a nucleic acid sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 98% or at least 99% homologous to the nucleic acid sequence set forth in SEQ ID NO:4. According to certain exemplary embodiments, the insertion within the *Psy1* gene comprises the nucleic acid sequence set forth in SEQ ID NO:4.

According to certain embodiments, the r^{arl} allele of *Psy1* comprises the nucleic acid sequence set forth in SEQ ID NO:5.

The present invention further provides methods for producing tomato cultivars having the *Arlecchino* phenotype, plants so produced and parts thereof.

According to a certain aspect, the present invention provides a method for producing a tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, the method comprising introducing into a tomato cultivar producing red fruit or a part thereof a genetic element comprising r^{arl} allele of *Phytoene synthase 1*, the r^{arl} allele comprises an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

Introduction of the genetic element into the genome of a selected tomato cultivar can be performed using any method as is known in the art.

Plant breeders and in particular seed companies use elite breeding lines, generally referred to as "elite lines" to provide a constant quality product. The elite lines are the result of intensive inbreeding and combine multiple superior characteristics such as high yield, fruit quality, resistance to pests and diseases, and tolerance to abiotic stress. The

average yield of these elite lines is generally much higher than the original wild accessions from which many of the modern tomato varieties are descendants. The elite lines can be used directly as crop plant, but are typically used to produce so-called F1 or single-cross hybrids, produced by a cross between two (homozygous or inbred) elite lines. The F1 hybrids thus combine the genetic properties of the two parents into a single plant. An additional benefit of hybrids is that they express hybrid vigor or heterosis, the phenomenon that hybrid plants grow better than either (inbred) parent and show higher yields.

Backcross or pedigree selection is one method by which breeders add desirable agronomic traits to their elite breeding lines. The method involves crossing the breeding line with a line that expresses the desirable trait followed by backcrossing offspring plants expressing the trait to the recurrent parent. As a result, the selection of an individual as a parent in a breeding program is based on the performance of its forebears. Such methods are most effective in breeding for qualitatively-inherited traits, i.e. traits which are present or absent.

Recurrent selection is an alternative breeding method for improving breeding lines and involves systematic testing and selection of desirable progeny followed by recombination of the selected individuals to form a new population. Recurrent selection has proven effective for improving quantitative traits in crop plants. Recurrent selection, however, decreases the rate of broadening genetic basis underlying the various traits in a breeding program, and its potential is therefore limited.

As disclosed herein, tomato plants producing yellow-red bicolor fruit can be produced by introducing a genetic element comprising r^{arl} allele of *Phytoene synthase 1*, into an elite breeding line.

Introducing the r^{arl} allele can be performed by plant breeding, i.e. by crossing a donor plant comprising the r^{arl} allele with a recipient plant, preferably an elite cultivar tomato plant not comprising the r^{arl} allele.

Alternatively, a nucleic acid, preferably DNA, comprising the r^{arl} allele may be isolated by any method known in the art and introduced into the genome of a tomato plant producing red fruit.

Transforming plants with isolated nucleic acid sequence generally involves the

construction of an expression vector that will function in plant cells. According to the teachings of the present invention, such a vector comprises a nucleic acid sequence that comprises the r^{arl} allele. Typically, the vector comprises the r^{arl} allele under control of or operatively linked to a regulatory element. According to certain embodiments, the regulatory element is selected from the group consisting of a promoter, an enhancer and a translation termination sequence. The expression vector may contain one or more such operably linked gene/alleles/regulatory element combinations, provided that at least one of the alleles contained in the combinations comprises the r^{arl} allele. The vector(s) may be in the form of a plasmid, and can be used, alone or in combination with other plasmids, in a method for producing transgenic plants that produce fruit with the *Arlecchino* phenotype, using transformation methods known in the art to be suitable for transforming nucleic acid sequences into tomato (dicotyledonous) plants.

Expression vectors can include at least one marker (reporter) gene, operably linked to a regulatory element (such as a promoter) that allows transformed cells containing the marker to be either recovered by negative selection (by inhibiting the growth of cells that do not contain the selectable marker gene), or by positive selection (by screening for the product encoded by the markers gene). Many commonly used selectable marker genes for plant transformation are known in the art, and include, for example, genes that code for enzymes that metabolically detoxify a selective chemical agent which may be an antibiotic or a herbicide, or genes that encode an altered target which is insensitive to the inhibitor. Several positive selection methods are known in the art, such as mannose selection. Alternatively, marker-less transformation can be used to obtain plants without mentioned marker genes, the techniques for which are known in the art.

Methods for transforming a plant cell with nucleic acids sequences according to the present invention are known in the art. As used herein the term “transformation” or “transforming” describes a process by which a foreign nucleic acid sequence, such as a vector, enters and changes a recipient cell into a transformed, genetically modified or transgenic cell. Transformation may be stable, wherein the nucleic acid sequence is integrated into the plant genome and as such represents a stable and inherited trait, or transient, wherein the nucleic acid sequence is expressed by the cell transformed but is not integrated into the genome, and as such represents a transient trait. According to

typical embodiments the nucleic acid sequence of the present invention is stably transformed into a plant cell.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (for example, Potrykus I. 1991. Annu Rev Plant Physiol Plant Mol Biol 42:205-225; Shimamoto K et al., 1989. Nature 338:274-276).

The principal methods of the stable integration of exogenous DNA into plant genomic DNA includes two main approaches:

Agrobacterium-mediated gene transfer: The *Agrobacterium*-mediated system includes the use of plasmid vectors that contain defined DNA segments which integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the *Agrobacterium* delivery system. A widely used approach is the leaf-disc procedure, which can be performed with any tissue explant that provides a good source for initiation of whole-plant differentiation (Horsch et al., 1988. Plant Molecular Biology Manual A5, 1-9, Kluwer Academic Publishers, Dordrecht). A supplementary approach employs the *Agrobacterium* delivery system in combination with vacuum infiltration. *Agrobacterium* mediated transformation protocols for tomato plants are known to a person skilled in the art.

Direct nucleic acid transfer: There are various methods of direct nucleic acid transfer into plant cells. In electroporation, protoplasts are briefly exposed to a strong electric field, opening up mini-pores to allow DNA to enter. In microinjection, the nucleic acid is mechanically injected directly into the cells using micropipettes. In microparticle bombardment, the nucleic acid is adsorbed on microprojectiles such as magnesium sulfate crystals or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues. Another method for introducing nucleic acids to plants is via the sonication of target cells. Alternatively, liposome or spheroplast fusion has been used to introduce expression vectors into plants.

Following transformation of tomato target tissues, expression of the above described selectable marker genes allows for preferential selection of transformed cells, tissues and/or plants, using regeneration and selection methods now well known in the art.

According to certain embodiments, the present invention provides a method for producing tomato plants with *Arlecchino* phenotype, comprising the steps of:

(a) introducing a genetic element comprising the r^{arl} allele from a donor tomato plant comprising the genetic element into a recipient tomato cultivar, preferably an elite cultivar to provide offspring cultivated tomato plants;

(b) examining a nucleic acid sample obtained from each offspring cultivated tomato plants for the presence of r^{arl} allele; and

(c) selecting cultivated tomato plants comprising said r^{arl} allele.

This method can be defined as "marker assisted selection" as the selection of the desired *Arlecchino* phenotype is performed using nucleic acid markers specific for the *Arlecchino* genotype. Since the *Arlecchino* phenotype can only be properly identified phenotypically when the plant has produced fruit, it is of particular advantage that the establishment of proper introgression of the genetic element in offspring plants may be monitored by using the gene specific markers.

Introducing the genetic element comprising the r^{arl} allele of P*sy1* into a recipient plant can be performed by any method as described hereinabove and is known in the art.

Any method for obtaining a genetic material from the offspring tomato cultivar and any suitable molecular marker as are known in the art can be used for selecting *Arlecchino* genotype according to the teachings of the present invention.

As used herein, the terms "molecular marker" or "molecular markers" refer to a molecular indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are diversity array technology (DArT) markers, restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), sequence-characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers, sequence-characterized hybridization markers; or any combination thereof. According to certain exemplary embodiments, the step of examining a nucleic acid sample obtained from each offspring cultivated tomato plants for the presence of r^{arl} allele comprise the use of a set of bi-directional primers. Bi-directional means that the orientation of the primers is such that one functions as the

forward and one as the reverse primer in an amplification reaction of nucleic acid. The bi-directional primers are typically used in an amplification reaction on genomic DNA that amplifies a unique nucleic acid sequence of the r^{arl} allele of *Psy1* or a marker thereof but that does not amplify the wild type *Psy1* allele. According to certain embodiments, the pair of primers is designed to amplify an r^{arl} allele marker comprising the nucleic acid sequence set forth in SEQ ID NO:7 (TCTGGATAATCTGGATA). According to certain exemplary embodiments, the r^{arl} allele marker is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:10 (CAGTGCCAGAAGAGGAAGA) and SEQ ID NO:11 (TTGCGGTACAAGACCAAAGA).

According to additional embodiments, the pair of primers is designed to amplify the full length transposon insertion. According to certain exemplary embodiments, the transposon is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:12 (GTGGATCCTGAAATGGCTTG) and SEQ ID NO:13 (AGTACTAATAAAATGGTTTTGCC).

According to yet additional embodiments, the pair of primers is designed to amplify the 3' genomic junction of the transposon insertion within the Psy1 allele. According to certain exemplary embodiments, the 3' transposon insertion junction is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:14 (GGGCTAGTCGGTGTATCAT) and SEQ ID NO:11 (TTGCGGTACAAGACCAAAGA).

According to yet further embodiments, the pair of primers is designed to amplify the 5' genomic junction of the transposon insertion within the *Psy1* allele. According to certain exemplary embodiments, the 5' transposon insertion junction is amplified by a pair of primer comprising the nucleic acid sequence set forth in SEQ ID NO:15 (CTGGAAGGGTGACCGATAAA) and SEQ ID NO:16 (ATGATACACCGACTAGCCC).

Additionally or alternatively, the markers are sequence specific probes that specifically hybridize under stringent conditions to the r^{arl} allele of *Psy1* but not to its wild type allele, and that can be detected thereafter by various methods as are well to a person skilled in the art.

Nevertheless, it is to be explicitly understood that the method aspects of the invention are not limited to the use of the markers identified herein, and that methods of the present invention may also make use of markers not explicitly disclosed herein or even yet to be identified, as identifying and using such markers is well within the skills of a person with knowledge in the Art.

In an additional or alternative method, the offspring cultivated tomato plants are phenotypically examined for the *Arlecchino* appearance as exemplifies hereinbelow. According to these embodiments, the offspring plants are grown to produce fruit and the fruit are examined for the presence of red and yellow sections throughout the fruit (the *Arlecchino* phenotype).

According to certain embodiments, the cultivar tomato plant having fruit with *Arlecchino* phenotype is an inbred plant. According to other embodiments, the cultivar tomato plant having fruit with *Arlecchino* phenotype is a hybrid plant. The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention. One skilled in the art can readily devise many variations and modifications of the principles disclosed herein without departing from the scope of the invention.

EXAMPLES

Example 1: Whole genome backcross inbred lines (BILs)

The *Solanum pimpinellifolium* BILs were constructed from a cross between a small red-fruited self-compatible accession of the wild species *Solanum pimpinellifolium* (designated LA1589) and the processing-tomato, inbred variety, cv. E6203 (TA209) (*S. lycopersicum*) (Grandillo S and Tanksley S D. 1996. Theor. Appl. Genet. 92: 935–951). During the construction of BIL from both species, early generations were evaluated for yield associated traits, as a part of the advanced backcross (AB) QTL studies (reviewed by Grandillo S et al., 2007. Theor. Appl. Genet. 92: 935–951) and also for various morphological traits and biochemical properties. The *S. pimpinellifolium* BILs are composed from 178 lines and the genetic map of this

resource was constructed from 4008, genome anchored, SNP markers that were found to be polymorphic between the wild species *S. pimpinellifolium* and the recurrent parent cv. E6203. The markers were divided into 873 bins with an average length of 0.87 Mbp/bin and composed an average of 4.59 SNPs/bin. Each of the bins showed a unique pattern of segregation, enabling the calculation of map distance in cM between pairs of neighboring bins. The calculated map is 1174.1 cM long and covers 100% of the wild species genome where the longest linkage group represents chromosome 3 (129.5cM) and the shortest represents chromosome 6 (60.2cM).

Example 2: The *Arlecchino* Mutation

The creation of the interspecific BIL population from *Solanum pimpinellifolium* and *Solanum lycopersicum* resulted in *de novo* formation of mutations within the BIL population. A single plant having an unstable fruit color phenotype was identified in BIL#112. The plant was produced during the year of 2010 (within the BC2self10 generation, Figure 1 and the observed phenotype has not been detected previously in the various variety collections.

This unique mutant was characterized by parallel color bands of yellow and red, which paint the fruit longitudinally on the external epidermis (Figure 2A) but importantly extends into the flesh (pericarp), seeds jelly and placenta (Figure 2B). Hitherto, many mutations resulting in yellow tomato fruit have been identified; however, none of the mutations show yellow-red stripes or segments throughout the fruit cross, disclosed herein for the first time. Another characteristic of this mutant is that there is no observable phenotypic effect on fruit epidermis color in the immature green stage and only upon maturity the phenotype is revealed. The mutation phenotype has been assigned the name "*Arlecchino*". The first *Arlecchino* phenotype discovered in 2010 was unstable along the plant and the inflorescence, particularly in that complete red fruit were observed together with *Arlecchino* fruit on the same plant.

The mode of inheritance of the *Arlecchino* phenotype was examined by crossing the plant identified in the BIL #112 with its parent cv. E6203 (TA209) and other wild type *Solanum lycopersicum* cultivars as compared to self-cross. The F1 hybrid plants had only normal red fruit while the BIL #112 self-progeny showed the characteristic *Arlecchino* phenotype. These results indicate that the new mutation is recessive. In the

F2 population derived from selfing the F1 hybrid, the following progeny was observed: 29 plants with red fruits; 9 plants with the *Arlecchino* phenotype; and 2 plants with completely yellow fruit (Total 40). Additional F2 population derived from an independent F1 hybrid produced 90 plants with complete red fruit; 22 with *Arlecchino* fruit; and 5 with complete yellow fruit (total of 117 plants) (Figure 3). The number of *Arlecchino* + yellow out of the total population fits the 1:3 ratio suggesting a single Mendelian gene with variable penetrance and expressivity.

To further examine the mode of the trait segregation, F3 progeny was examined. The F3 progeny was formed from selfing 31 F2 plants having red (21plants), Arlecchino (8 plants) and yellow (2 plants) fruit phenotype. About 40 plants of the F3 generation of each F2 cross (31 x 40) were examined. The plants were phenotyped for fruit color and the genotype of the F2 plant was derived based on the progeny tested. The results indicate that 13 plants out of 21 red F2 plants segregated for the fruit phenotype at a ratio of 25% *Arlecchino* and 75% red (Table 1). The other eight red fruit F2 plants did not segregate in their F3 progeny meaning that all the F3 plants were red (the F3 of Plant 4853-26 showed only a single *Arlecchino* phenotype and thus it was assumed to be a contaminant and therefore this line was scored as homozygous; Table 1). The two yellow-phenotype F2 plants did not segregate and all the F3 progeny plants had completely yellow fruits. The 8 *Arlecchino* F2 plant did not show any consistent segregation ratio. All F3 progeny of line No. 4853-27 showed the *Arlecchino* phenotype, indicating this line as a stable *Arlecchino* parent.

Table 1: Summary of F3 Progeny Tests

		F3 Phenotype (Fruit Color)				
Line	F2 Phenotype (Fruit Color)	Red	*Arlecchino*	Yellow	Total	Estimated genotype
4583-1	Red	33	7	0	40	Heterozygous
4583-2	*Arlecchino*	14	16	0	30	
4583-3	Red	15	0	0	15	Homozygous
4583-4	Red	40	0	0	40	Homozygous
4583-5	*Arlecchino*	22	7	0	29	
45836	Red	28	8	0	36	Heterozygous
4583-7	Red	37	0	0	37	Homozygous
4583-8	Red	26	6	0	32	Heterozygous

		F3 Phenotype (Fruit Color)				
Line	F2 Phenotype (Fruit Color)	Red	*Arlecchino*	Yellow	Total	Estimated genotype
4583-9	Red	22	0	0	22	Homozygous
4583-10	Red	30	9	0	39	Heterozygous
4583-11	Red	40	0	0	40	Homozygous
4583-12	Red	32	8	0	40	Heterozygous
4583-13	Red	14	7	0	21	Heterozygous
4583-14	Red	34	6	0	40	Heterozygous
4583-15	Yellow	0	0	40	40	
4583-16	Red	30	10	0	40	Heterozygous
4583-17	*Arlecchino*	19	13	0	32	
4583-18	Red	40	0	0	40	Homozygous
4583-19	Red	36	4	0	40	Heterozygous
4583-20	Red	22	8	0	30	Heterozygous
4583-21	Red	25	0	0	25	Homozygous
4583-22	*Arlecchino*	14	21	0	35	
4583-23	Red					
4583-24	Red	12	4	0	16	Heterozygous
4583-25	Yellow	0	0	35	35	
4583-26	Red	32	1*	0	32	Homozygous
4583-27	*Arlecchino*	0	16	0	16	
4583-28	*Arlecchino*	5	26	0	31	
4583-29	Red	19	6	0	25	Heterozygous
4583-30	Red					
4583-31	Red	24	11	0	35	Heterozygous
4583-32	Red					
4583-33	Red					
4583-34	*Arlecchino*	21	9	0	30	
4583-35	*Arlecchino*	32	8	0	40	
4583-36	Red					
4583-37	Red					
4583-38	Red					
4583-39	*Arlecchino*					
4583-40	Red					

Example 3: Allelic configuration of the *Arlecchino* phenotype

The results described above indicated that the *Arlecchino* phenotype is recessively inherited as fruit of F1 plants derived from the cross of BIL#112 and TA209 and other wild type (WT) cultivars were found to be completely red. Phenotypic complementation was also found in F1 plants derived from crosses of BIL#112 to the *tangerine* mutation

e3406m2 and the *zeta* mutation e2083m1. Only in crosses of plant having the *Arlecchino* phenotype two independent mutations having the phenotype *yellow flesh* (*r/r*; defective in the *Phytoene synthase 1* gene (*Psy1*) showed lack of complementation and the fruit showed a mild stripped phenotype (Table 2). These results suggest that *Arlecchino* is possibly allelic to *yellow flesh*.

Table 2: Allelism tests of the Arlecchino phenotype

Line	E6203 (TA209) (R/R)	M82 (R/R)	e3756m2 (r/r)	LA2997 (r/r)	e3406m2 (t/t)	e2083m1 (*zeta/zeta*)
BIL 112 *Arlecchino*	Red	Red	Weak Arlecchino	Weak Arlecchino	Red	Red

Example 4: Analysis of carotenoid content in *Arlecchino*-phenotype fruit

The carotenoid content within the red and yellow sections of ripe fruit having *Arlecchino* phenotype was examined and compared to the carotenoid content within red fruit of wild type tomato (M82) and yellow fruit of the *yellow flesh* variety e3756m2 (r/r; M82 EMS derivative). The carotenoids were extracted from the different section as described in Kachanovsky et al. (Kachanovsky D E et al., 2012. Proc. Natl. Acad. Sci. 109:19021–19026) and separated using high performance liquid chromatography (HPLC) using Waters 996 photodiode array detector (Ronen G et. al., 1999. Plant J 17:341–351). Red sections within the *Arlecchino* fruit have about 5 times higher total carotenoid content with a profile similar to wild type fruit while the yellow sections carotenoid content is significantly lower with a profile similar to the *yellow flesh* (e3756m2) mutant fruit (Figure 4, Table 3).

Table 3: Carotenoid composition in red and yellow sections of ripe *Arlecchino* fruit (µg/g fresh weight)

	Arlecchino Red section	*Arlecchino* Yellow sections
Phytoene	6.57±2.42	0.19±0.04
phytofluene	4.13±1.96	0.14±0.05
trans-Lycopene	7.96±7.18	1.46±0.88
β-Carotene	2.79±1.06	0.29±0.99
Lutein	1.13±0.30	0.76±0.15
tri-cis-ζ-carotene	0.40±0.20	-

	Arlecchino Red section	*Arlecchino* Yellow sections
di-cis-ζ-carotene	0.42±0.20	-
Others	1.53±1.07	1.14±0.24
Total carotenoids	26.7±4.42	5.24±2.38

Example 5: Sequence analysis of the *Arlecchino*-phenotype mutation

Carotenoids are 40-carbon isoprenoid pigments synthesized by all plants, algae and cyanobacteria as well as by several non-photosynthetic bacteria and fungi. The polyene chain of carotenoids may extend from 3 to 15 conjugated double bonds, which are responsible for the carotenoid characteristic absorption spectra and confer specific photochemical properties. The first committed step in the carotenoid pathway is the head to head condensation of two geranylgeranyl pyrophosphate (GGPP) molecules to produce phytoene, the first C40 carotenoid, catalyzed by the enzyme phytoene synthase (PSY). Initial DNA sequencing of *Phytoene synthase 1* in the original BIL#112 *Arlecchino* phenotype plant revealed a 9 bp insertion in intron 8 (ATCTGGATA, SEQ ID NO:1) that was inserted after nucleotide 3338.

Initial sequence analysis of the eight's intron in yellow and red sections of *Arlecchino* fruit

The eight's intron of *Psy1* was amplified by PCR from DNA samples obtained from red and yellow sections of fruit of *Arlecchino* phenotype and cloned into pGEM plasmid vector. Genomic DNA was extracted using a Genomic Plant DNA Purification Kit (Thermo). The intron was amplified using the primers listed in Table 4 below. *E. coli* cells were tranfected with pGEM plasmids carrying PCR products from yellow sectors or from red sectors. Seven *E. coli* colonies with pGEm clones from yellow tissue and 21 colonies with pGEM clones from the red tissues were tested. All DNA clones from yellow tissue showed the same sequence pattern of a duplication of the direct repeats of TCTGGATA (SEQ ID NO:2) separated by Adenine ("A) nucleotide (SEQ ID NO:7) designated herein as "the *Arlecchino* marker". The clones from the red tissues showed sequence variability amongst the colonies, with majority of the colonies containing transposon excision footprints while the rest of the colonies showing the same pattern as observed in the yellow colonies (Figure 5). These results indicated a possibility of an excision of a transposon from one copy or by contamination of yellow

cells in the red section.

Table 4: Primer pair for detecting the *Arlecchino* marker

Primer designation	Sequence	SEQ ID NO.
Primer ARL4 forward	5'- CAGTGCCAGAAGAGGAAGA -3'	10
Primer ARL4 reveres	5'- TTGCGGTACAAGACCAAAGA -3'	11

Determining the complete sequence of the r^{arl} allele

Initial PCR analysis confirmed the presence of the *Arlecchino* marker. PCR was conducted using READYMIX kit (Syntezza), 50-100 ng of genomic DNA, 0.4 µM of the Forward and Reverse primers listed in Table 4 hereinabove. PCR was initiated using a denaturation step at 95°C for 2 min, followed by 38 cycles of 45 s denaturation at 96°C, 30 s annealing at 58-60°C, and 90 s extension at 72°C, and finally 10 m extension at 72°C.

To sequence the full length of *Psy1* transcript from *Arlecchino* red and yellow fruits sections, 3'RACE was executed. RNA was extracted from yellow and red sectors of *Arlecchino* fruits by Thermo scientific GeneJET plant RNA purification Mini Kit #K0801. RNA was treated with Dnase I (New England BioLabs #M0303L) and then reverse transcribed by M-Mulv Reverse Transctiptase (New Englands BioLabs #M0253L) using an Oligo-dT-adaptor primer.

To amplify the *Psy1* transcripts a PCR reaction was performed using a specific primer for *Psy1* and an adaptor primer (Table 5).

Table 5: Primers used in 3'RACE assay

Primer description	Sequence	SEQ ID NO.
Oligo-dT-adaptor primer	ctgtgaatgctgcgactacgatT(X20)	17
Adaptor primer	ctgtgaatgctgcgactacgat	18
Psy1 specific primer	AACTTGTTGATGGCCCAAAC	19

Amplified *Psy1* transcripts were cloned into pJET library (Thermo Scientific CloneJET PCR Cloning Kit).

Three spliced variants were detected in colonies obtained from *Arlecchino* fruit yellow sections, while in colonies obtained from red sections wild type transcript of *Psy1* was detected in addition to the mutated transcripts. Two of the transcripts were found to be fused to a short part from the last intron and the third transcript was found to be fused to 400bp sequence of a known hAT super-family transposon. In all three variants, the last exon (exon 9) was missing (Figure 6).

Based on the above analysis, several primer pairs were designed (Table 6). Genomic DNA was extracted from young leaves or yellow fruit sections of plants having the *Arlecchino* phenotype using a Genomic Plant DNA Purification Kit (Thermo). PCR was conducted using a 50ng-100ng of genomic DNA, PCR buffer, 2.5 mM dNTPs, 0.2μM-0.3μM of the Forward and Reverse primers, and 0.5 units of PrimeSTAR GXL DNA Polymerase (Takara Bio). PCR was initiated using a denaturation step at 98°C for 3 min, followed by 38 cycles of 10 seconds denaturation at 96°C, 15 seconds annealing at 55-60°C, and 180 seconds extension at 68°C, and finally 90 seconds extension at 68°C. These reactions enabled the amplification of the full length transposon as well of the area of the *Psy1* adjacent to the intron, as described in Figure 8.

Table 6: Primer pairs for detecting the r^{arl} allele

Primer designation	Sequence	SEQ ID NO.	Purpose
Primer ARL1 Forward	5'-GTGGATCCTGAAATGGCTTG-3'	12	Amplification of full length of transposon
Primer ARL1 Reverse	5'-AGTACTAATAAAATGGTTTTGCC-3'	13	
Primer ARL2 Forward	5'-GGGCTAGTCGGTGTATCAT-3'	14	Amplification of 3'genomic junctions of the transposon
Primer ARL2 Reverse	5'-TTGCGGTACAAGACCAAAGA-3'	11	
Primer ARL3 Forward	5'-CTGGAAGGGTGACCGATAAA-3'	15	Amplification of 3'genomic junctions of the transposon
Primer ARL3 Reverse	5'-ATGATACACCGACTAGCCC-3'	16	

Example 6: Sequence analysis of *Psy1* transcript in leaves and fruit yellow and red sections of *Arlecchino* plants

Psy1 transcript was amplified from RNA extracted from *Arlecchino* red and yellow fruit sections and *Arlecchino* leaf tissue using three pairs of primers. Reaction I, aimed at amplifying a segment stretching from exon 7 to exon 8 was successful in all three RNA samples examined. Reaction II and III, aimed at amplifying of a segment stretching from exon 7 to exon 8 and from exon 8 to exon 9, respectively, were successful only in samples containing RNA extracted from *Arlecchino* fruit red sections (Table 7). These results indicate that exon 9 is impaired in yellow and leaf tissue.

Table 7: Amplification of *Psy1* transcript using three different primer pairs

	Forward primers:	Reverse primers:	Area amplified
Reaction I	Psy1_f2 AGCCATTCAGAGATATGATTGA (SEQ ID NO:20)	Psy1-4 rev ATCGGATAGACCTGCCTGTG (SEQ ID NO:21)	Exon7-Exon8
Reaction II	psy1_f2 AGCCATTCAGAGATATGATTGA (SEQ ID NO:20)	Psy1_r2 TTATCTTTGAAGAGAGGCAGT (SEQ ID NO:22)	Exon7-Exon9
Reaction III	ARL3 CTGGAAGGGTGACCGATAAA (SEQ ID NO:15)	Psy1_r3 GATAAAGTGAAGATACAACAAC (SEQ ID NO:23)	Exon8-Exon9

Example 7: The *Arlecchino* transposon

Arlecchino transposon sequence was completed using the GXL polymerase and primers:

ARL1 Forward: GTGGATCCTGAAATGGCTTG (SEQ ID NO:12);

ARL1 Reveres: AGTACTAATAAAATGGTTTTGCC (SEQ ID NO:13); and

ARL3 Reveres: ATGATACACCGACTAGCCC (SEQ ID NO:16).

The transposon comprises 3903 nucleic acids (SEQ ID NO:3) and with inverted repeats at the 5' and 3'ends providing for its insertion into *Psy1* (Figure 7).

Sequencing of the *Arlecchino* transposon showed that it contains an open reading frame similar to known transposases (such as in Tam3 from *Antirrhinum majus*) which contains a dimerization domain and a Zinc finger-DNA binding domain and thus is

potentially an autonomous element (SEQ ID NO:24). The nucleic acids sequence of the *Arlecchino Psy1* gene (SEQ ID NO:5) is presented in Figure 8.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

CLAIMS

1. A tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to at least one allele of r^{arl}, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

2. The tomato cultivar of claim 1, wherein cells of the yellow segments are homozygous for the r^{arl} allele.

3. The tomato cultivar of any one of claims 1-2, wherein the insertion comprises a transposon flanked by the nucleic acid sequence set forth in SEQ ID NO:2 at the transposon 3' end.

4. The tomato cultivar of any one of claims 1-2, wherein the insertion comprises a transposon flanked by the nucleic acid sequence set forth in SEQ ID NO:1 at the transposon 3'end.

5. The tomato cultivar of any one of claims 1-4, wherein the transposon comprises a nucleic acid sequence at least 90% homologous to the nucleic acid sequence set forth in SEQ ID NO:3.

6. The tomato cultivar of claim 5, wherein the transposon comprises the nucleic acid sequence set forth in SEQ ID NO:3.

7. The tomato cultivar of any one of claims 1-4, wherein the insertion within the *Psy1* allele comprises a nucleic acid sequence at least 90% homologous to the nucleic acid sequence set forth in SEQ ID NO:4.

8. The tomato cultivar of any one of claims 1-3, wherein the insertion within the *Psy1* allele comprises the nucleic acid sequence set forth in SEQ ID NO:4.

9. The tomato cultivar of claim 8, wherein the r^{arl} allele comprises the nucleic acid sequence set forth in SEQ ID NO:5.

10. The tomato cultivar of any one of claims 1-9, wherein the tomato cultivar comprises at least one pericarp cell homozygous for the r^{arl} allele and at

least one pericarp cell comprising at least one wild type *Psy1* allele or at least one *Psy1* allele comprising transposon excision footprint.

11. The tomato cultivar of claim 10, wherein the transposon excision footprint comprises at least one nucleotide deletion within the nucleic acid sequence set forth in SEQ ID NO:7.

12. The tomato cultivar of any one of claims 1-11, wherein the fruit is ripening fruit at the breaker stage and onward.

13. The tomato cultivar of any one of claims 1-12, said cultivar produces small "cherry" fruit.

14. The tomato cultivar of any one of claims 1-13, said cultivar is *Solanum lycopersicum*.

15. The tomato cultivar claim 1, said cultivar further comprises within its genome additional *Psy1* mutant allele encoding for a *yellow flesh* phenotype.

16. The tomato cultivar of claim 15, wherein the additional *Psy1* mutant allele comprises the nucleic acid sequence set forth in any one of SEQ ID NO:8 and SEQ ID NO:9.

17. The tomato cultivar of any one of claims 15-16, wherein cells of the yellow segments comprise one r^{arl} allele and one *Psy1* mutant allele encoding for the *yellow flesh* phenotype and the red segments comprise wild type *Psy1* allele, *Psy1* allele comprising transposon excision footprint or a combination thereof.

18. A tomato cultivar homozygous to the r^{arl} allele, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion of a transposon flanked by the nucleic acid sequence set forth in SEQ ID NO:2 at the transposon 3' end, wherein the tomato cultivar produces entirely yellow fruit.

19. The tomato cultivar of claim 18, wherein the r^{arl} allele comprises the nucleic acid sequence set forth in SEQ ID NO:5.

20. A seed of the tomato cultivar of any one of claims 17-18, wherein a plant

grown from the seed is homozygous for the r^{arl} allele and produces entirely yellow fruit.

21. A seed of the tomato cultivar of any one of claims 1-17, wherein a plant grown from the seed produces fruit having a phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to at least one allele of r^{arl}, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

22. A plant part of the tomato cultivar of any one of claims 1-21, the plant part is selected from the group consisting of leaves, pollen, embryos, roots, root tips, anthers, flowers, isolated cells, isolated tissues and any part thereof.

23. A tomato fruit having a phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, wherein the phenotype is linked to at least one allele of r^{arl} in a cultivated tomato plant producing the fruit, the r^{arl} allele is *Phytoene synthase 1* (*Psy1*) allele comprising an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

24. The tomato fruit of claim 23, wherein the r^{arl} allele comprises the nucleic acid sequence set forth in SEQ ID NO:5.

25. The tomato fruit of any one of claims 23-24, wherein cells of the yellow segments are homozygous for the r^{arl} allele.

26. A method for producing a tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, the method comprising introducing into a tomato cultivar producing red fruit or a part thereof a genetic element comprising r^{arl} allele of *Phytoene synthase 1*, the r^{arl} allele comprises an insertion within an intron of the allele, wherein the insertion results in a non-functional splice variant of *Psy1*.

27. The method of claim 26, wherein the genetic element is introduced by

crossing the tomato cultivar producing red fruit with a donor tomato plant comprising the genetic element to provide offspring cultivated tomato plants.

28. The method of claim 27, said method further comprises the steps of:

a. examining a nucleic acid sample obtained from each offspring cultivated tomato plant or par thereof for the presence of r^{arl} allele;

b. selecting offspring cultivated tomato plants comprising the r^{arl} allele; and

c. examining the fruit produced by the plants selected in step (b) and electing cultivated tomato plants producing fruit with *Arlecchino* phenotype.

29. The method of claim 26, wherein the genetic element is introduced by transforming a plurality of cells of the tomato cultivar producing red fruit with said genetic element.

30. The method of claim 29, said method further comprises:

a. examining a nucleic acid sample obtained from each transformed cell for the presence of r^{arl} allele;

b. selecting a plurality of cells comprising the r^{arl} allele;

c. regenerating the plurality of transformed cells to obtain a plurality of transgenic plants comprising the r^{arl} allele; and

d. examining the fruit produced by the transgenic plant and selecting plant producing fruit having the *Arlecchino* phenotype.

31. The method of any one of claim 28 and 30, wherein examining the nucleic acid sample for the presence of the r^{arl} allele is performed by amplifying an r^{arl} allele marker comprising the nucleic acid sequence set forth in SEQ ID NO:7.

32. The method of claim 31, wherein the r^{arl} allele marker is amplified using a pair of primers comprising the nucleic acid sequence set forth in SEQ ID NO:10 and SEQ ID NO:11.

33. The method of any one of claims 28 and 30, wherein examining the nucleic acid sample for the presence of the r^{arl} allele is performed by amplifying a transposon sequence having the nucleic acids sequence set forth in SEQ ID NO:3.

34. The method of claim 33, wherein the transposon is amplified using a pair of primers comprising the nucleic acid sequence set forth in SEQ ID NO:12 and SEQ ID NO:13.

35. The method of any one of claims 28 and 30, wherein examining the nucleic acid sample for the presence of the r^{arl} allele is performed by amplifying the 3' genomic junction of a transposon insertion within the *Psy1* allele.

36. The method of claim 35, wherein the 3' genomic junction is amplified using a pair of primers comprising the nucleic acid sequence set forth in SEQ ID NO:14 and SEQ ID NO:11.

37. The method of any one of claims 28 and 30, wherein examining the nucleic acid sample for the presence of the r^{arl} allele is performed by amplifying the 5' genomic junction of a transposon insertion within the *Psy1* allele.

38. The method of claim 37, wherein the 5' genomic junction is amplified using a pair of primers comprising the nucleic acid sequence set forth in SEQ ID NO:15 and SEQ ID NO:16.

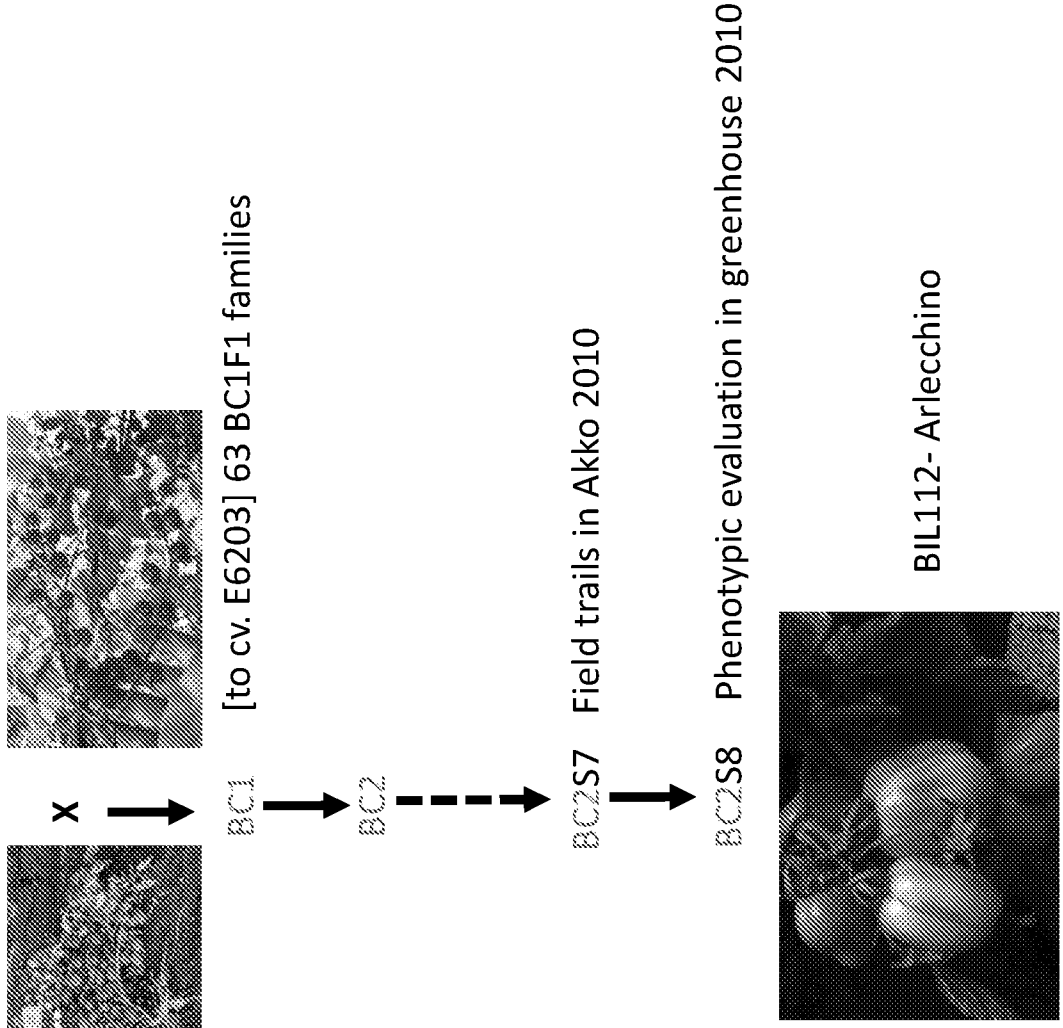
39. A method for producing a tomato cultivar producing fruit having an *Arlecchino* phenotype of yellow-red segments spanning from the placenta and/or locules across the fruit pericarp to the epidermis, the method comprises mutating at least one allele of *Phytoene synthase 1* by an insertion mutation.

40. The method of claim 39, wherein the insertion is within an intron resulting in a non-functional *Psy1* allele.

41. The method of claim 40, wherein the insertion is of a transposon.

42. An isolated polynucleotide encoding a mutated *Phytoene synthase 1* comprising the nucleic acids sequence as set forth in SEQ ID NO:5.

FIGURE 1


S. lycopersicum cv. E6203
x
S. pimpenellifolium
BC1
[to cv. E6203] 63 BC1F1 families
BC2
BC2S7
Field trails in Akko 2010
BC2S8
Phenotypic evaluation in greenhouse 2010
BIL112- Arlecchino

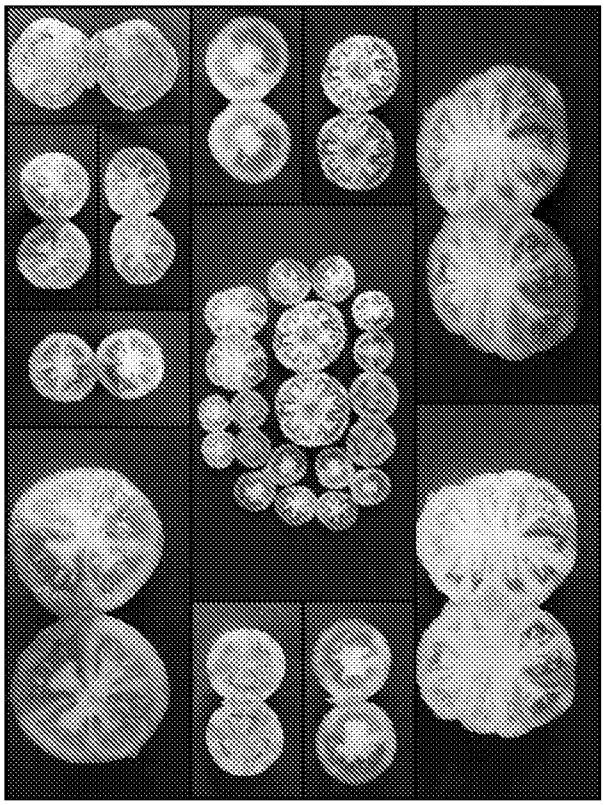


FIGURE 2B

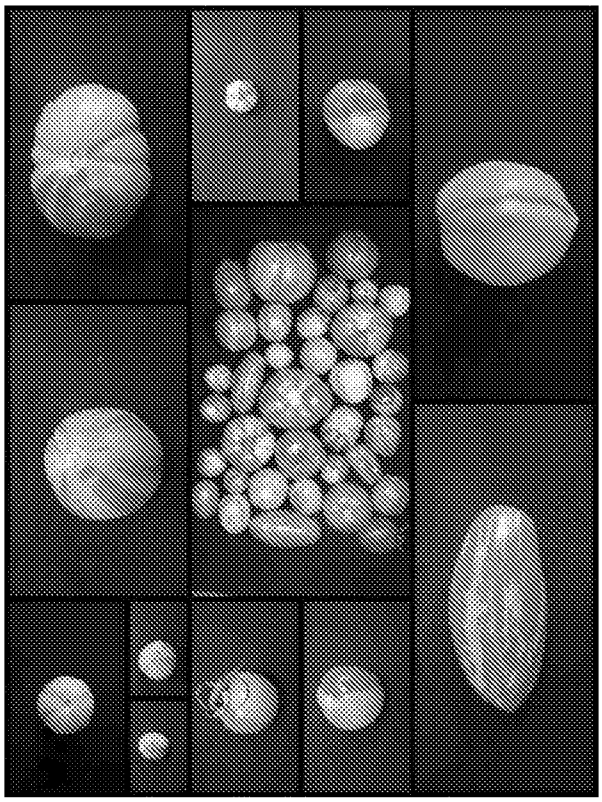


FIGURE 2A

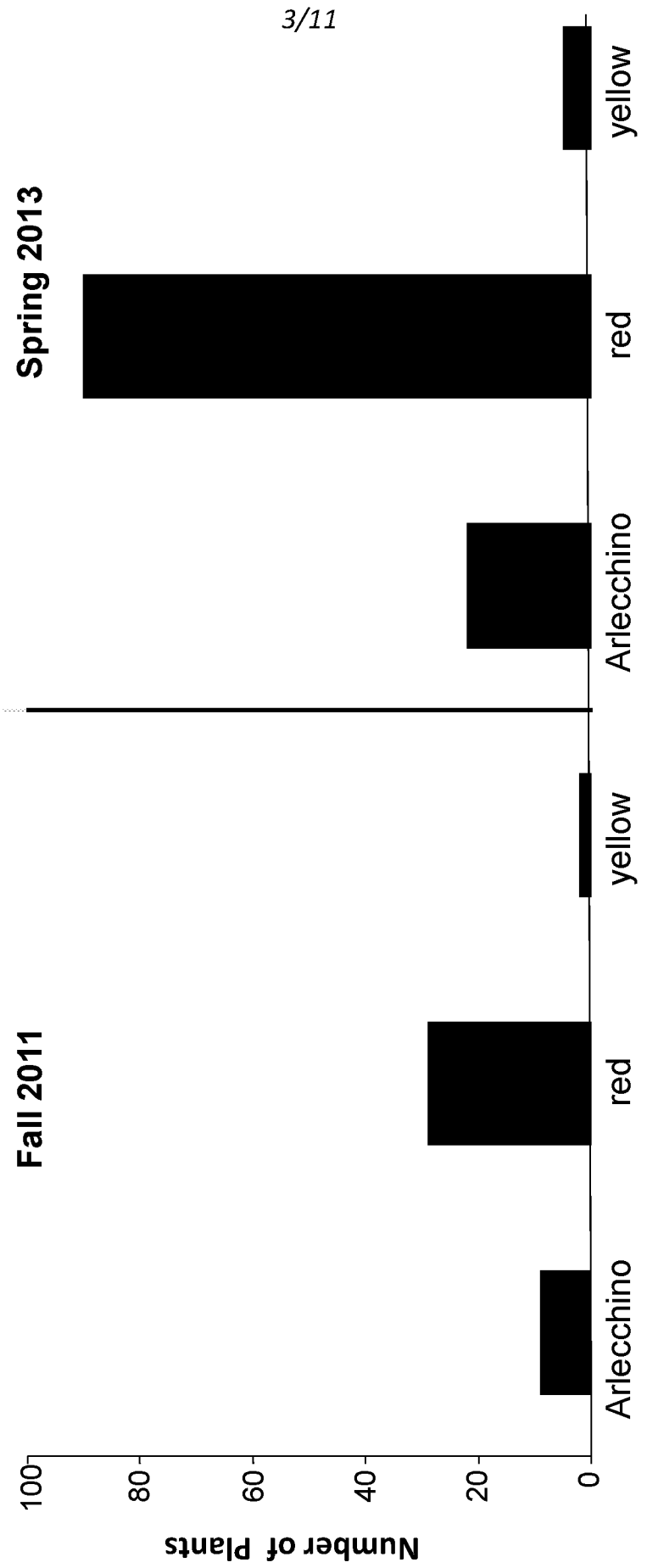

Spring 2013
Fall 2011
Number of Plants
100
80
60
40
20
0
Arlecchino
red
yellow
Arlecchino
red
yellow
Fruit color

FIGURE 3

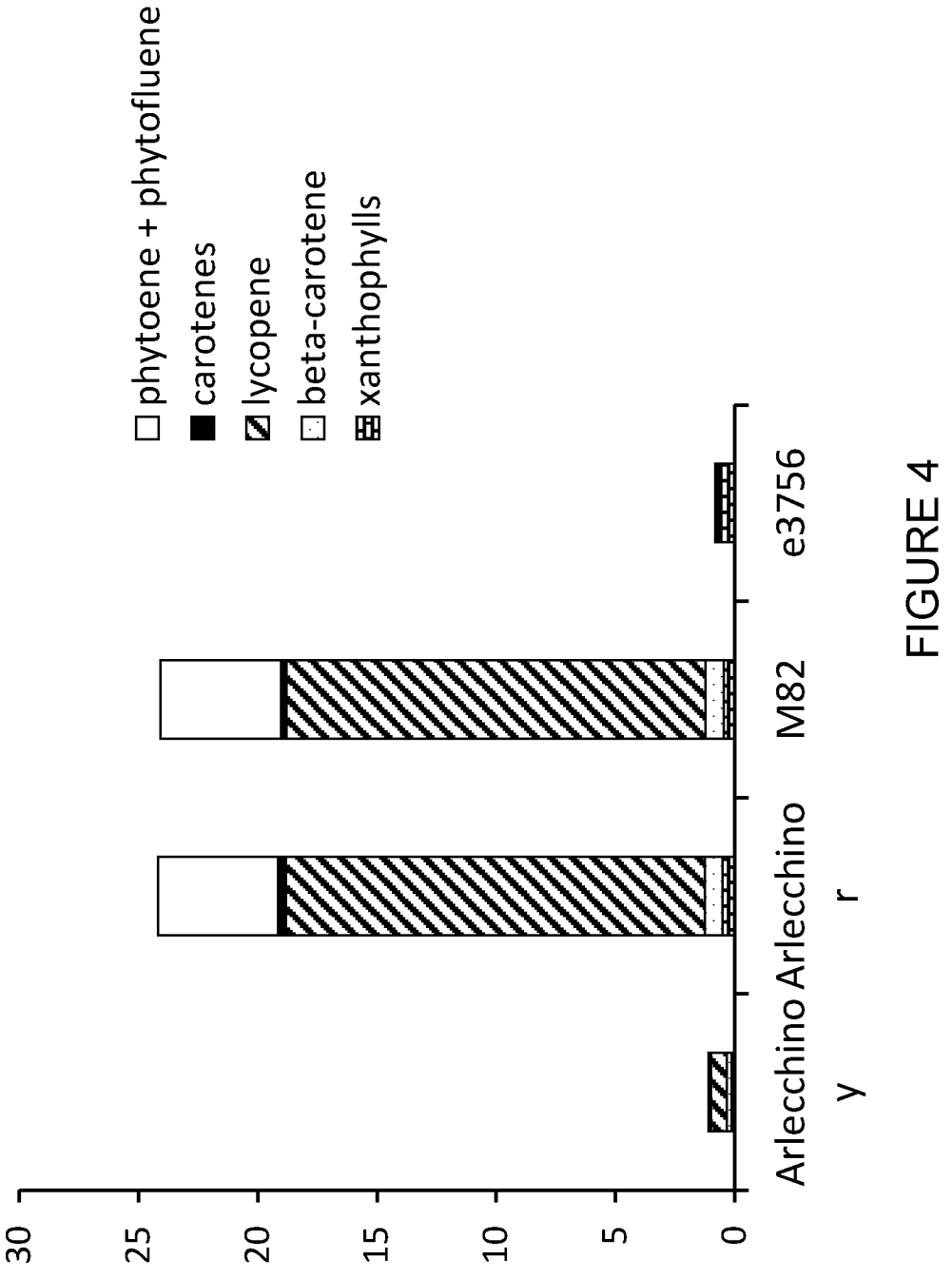

phytoene + phytofluene
carotenes
lycopene
beta-carotene
xanthophylls
30
25
20
15
10
5
0
Arlecchino
y
Arlecchino
r
M82
e3756

FIGURE 4

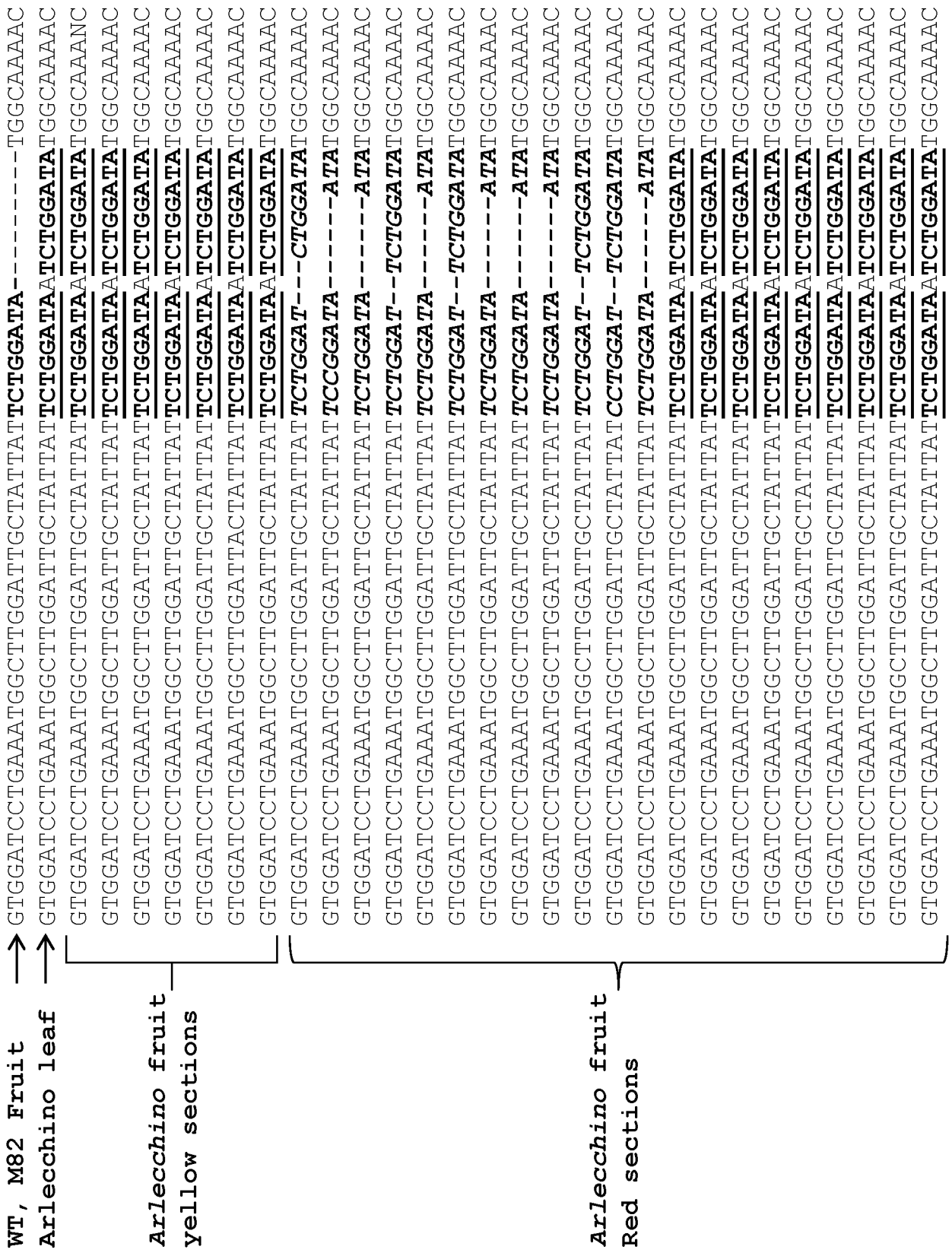

WT, M82 Fruit
Arlecchino leaf
Arlecchino fruit yellow sections
Arlecchino fruit Red sections

FIGURE 5

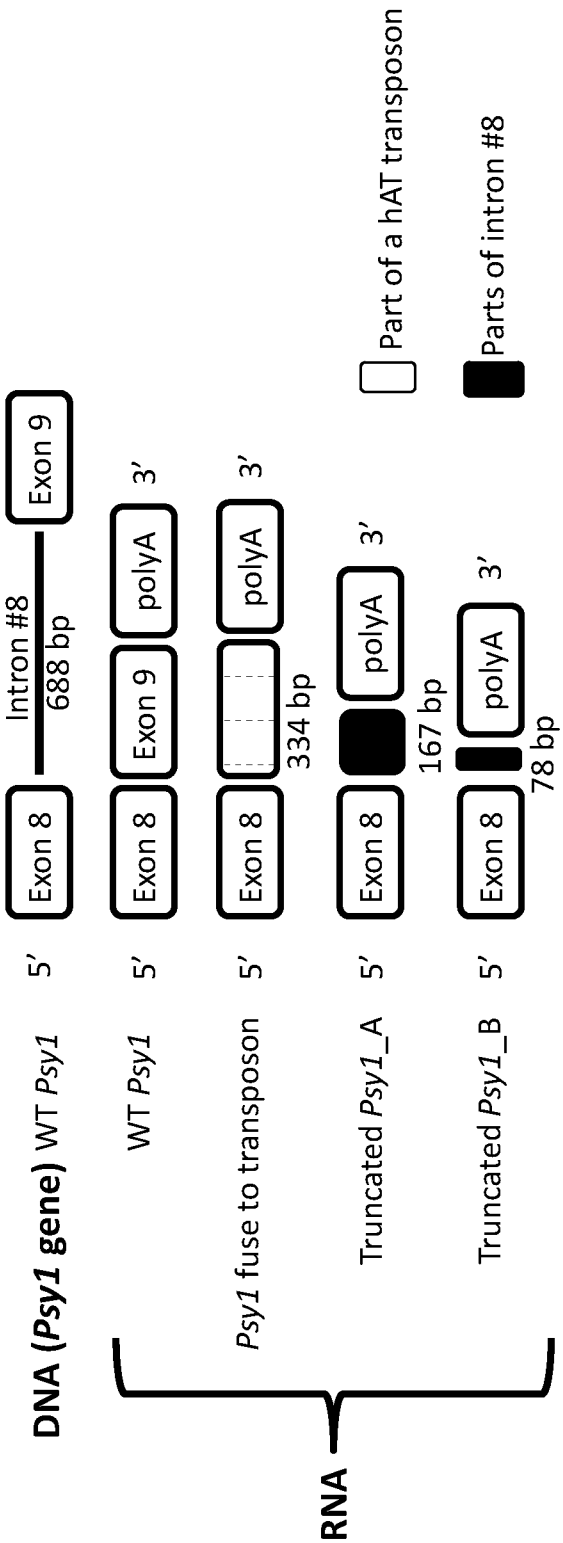

DNA (Psy1 gene)
WT Psy1
5'
Exon 8
Intron #8
688 bp
Exon 9
RNA
WT Psy1
5'
Exon 8
Exon 9
polyA
3'
Psy1 fuse to transposon
5'
Exon 8
334 bp
polyA
3'
Truncated Psy1_A
5'
Exon 8
167 bp
polyA
3'
Truncated Psy1_B
5'
Exon 8
78 bp
polyA
3'
Part of a hAT transposon
Parts of intron #8

FIGURE 6

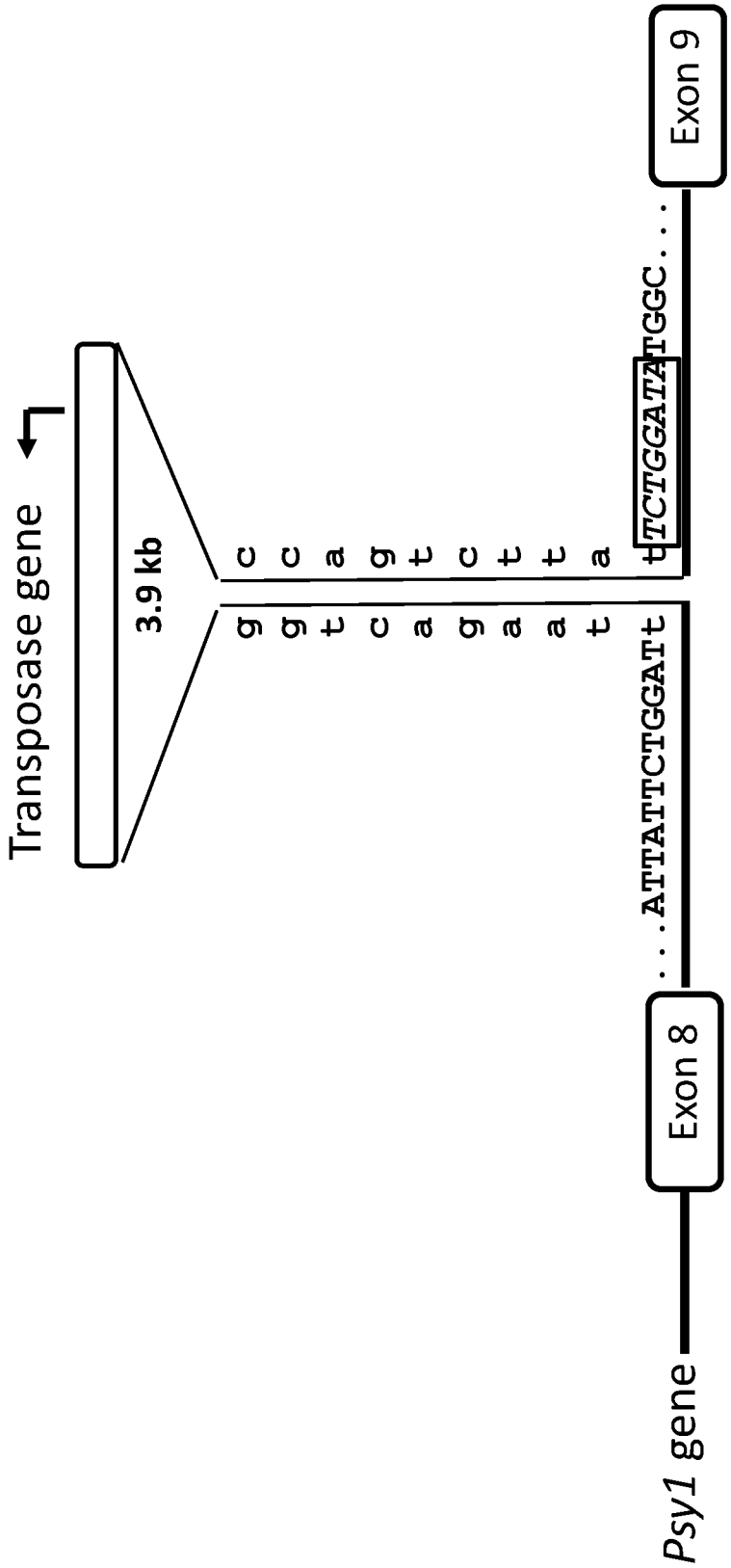


FIGURE 7A

Marker of *Arlecchino*

Sequence of PCR-amplified *Psy1*

GAAAT**TCTGGATA** A **TCTGGATA**TGGC

FIGURE 7B

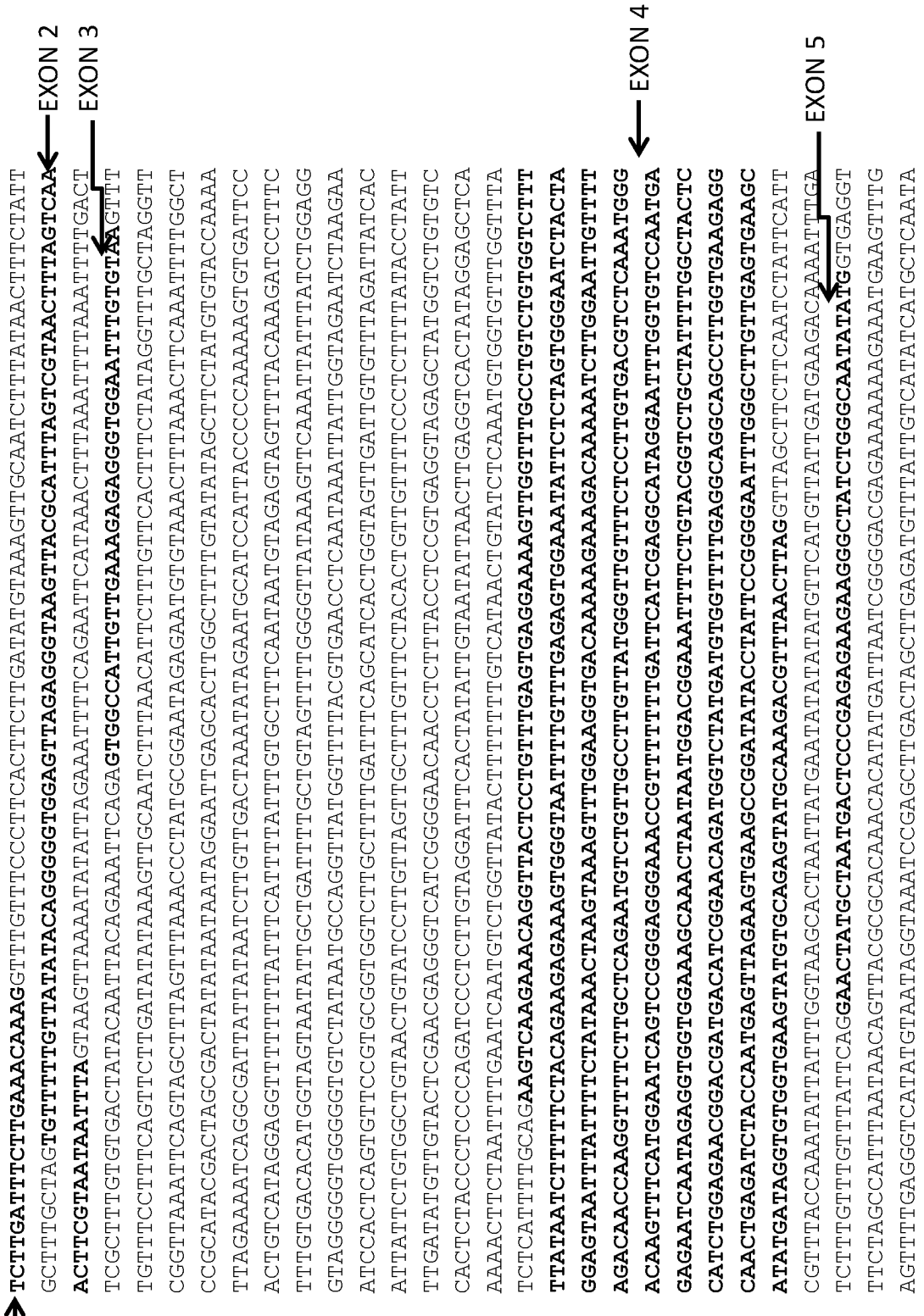


FIGURE 8

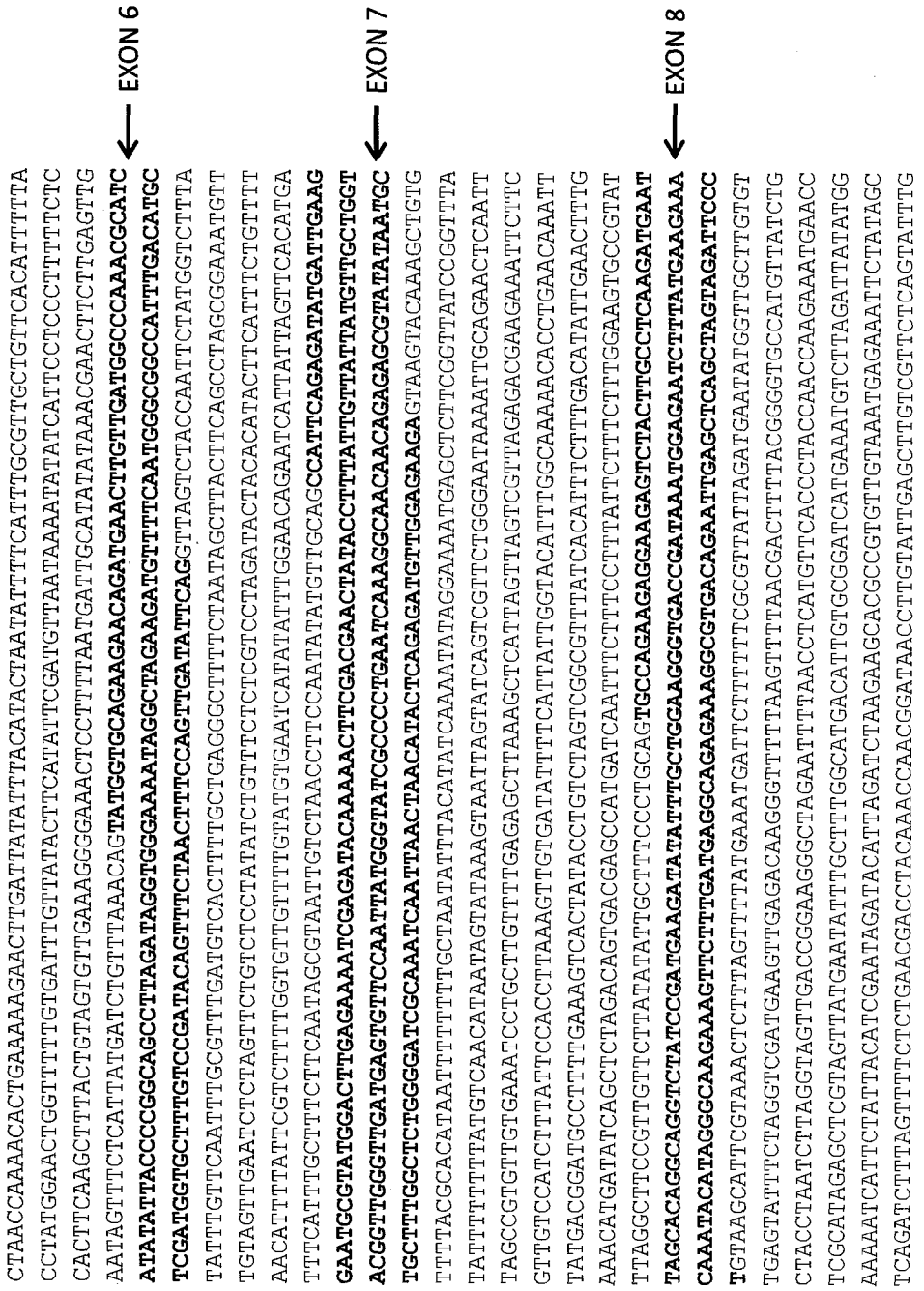


FIGURE 8 (Cont. 1)

CACTAACATTACGTCGTGTGGATCCTGAAATGGCTTGGATTGCTATTATTCTGGAT**ttaagactgg**tcaacggac
cggaaccgggtcaccggaccggaatgaaccggtaccggaccggaccggaatccgttgaccggaattttgaccggt
accgggatgaaccggaccggaattaccgggatggttcatcggttccgtcccgttccactatataccgggacggaa
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atttaatttatttaaatttgtaacaaattacaatttcaatttacaactattagtagagtacatagcttacgcagc
caccttctaggaagggttctgtttcaactatgtctcgaatgtaatactaatagttgtatagtcccgtaaatcctc
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tgtggtctccaatttgaaatcttgccgcgctgaaagctgcctccgaactactgatgatccttgaattgcaagcac
atccttcaccatcctactaagttttggatattgagctccacgatttctccaccagttcaatagttccggtatacc
attatcgtttgtaatatcatctgtaccctgttcaagatatttaacaaattcacatttattagaagagtcaagacc ⟵ Transposon
aagtttatgtttcactcgtccatgagcacctacttgatttgtagacgtttgaggattttcaacattatctaagaa
tgaatatttatcatacatttcttttgcaaagtattttatactattttgacatgttaccaaatcaggttcttcttc
aggttgaatatctaaattctgataaatgcattcaactaaagcttttgtaccatattctttatattcggggttgaa
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tacttcatcccaatcactatcattaattttaaattcaggataagcattatgattattaaatagcgtagtaatagg
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tatatgcgcggcacatctaacatgaaaagcatatttactaataggacaaagtctaggttctagcaaattaatagc
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FIGURE 8 (Cont. 2)

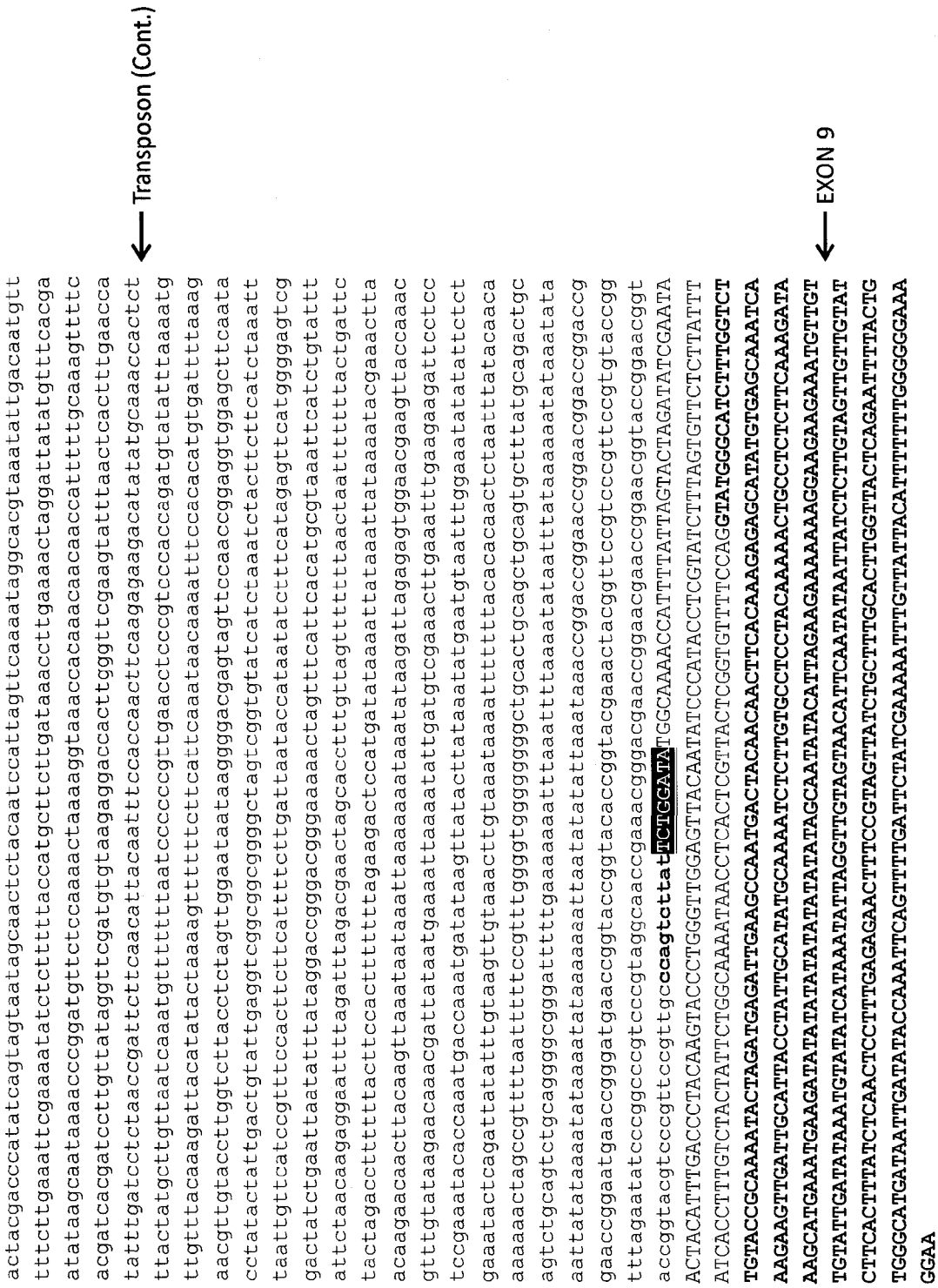

FIGURE 8 (Cont. 3)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2016/050719

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H5/08 C12Q1/68 C12N9/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A01H C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Irinas Tomaten & Kräuter: "Orange Russian 117", , 5 October 2008 (2008-10-05), XP002763604, Retrieved from the Internet: URL:http://www.irinas-shop.de/orange-russian-117-p-529.html [retrieved on 2016-10-27] the whole document ----- -/--	1-12,14, 21-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 2016	21/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bilang, Jürg

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D. E. KACHANOVSKY ET AL: "Epistasis in tomato color mutations involves regulation of phytoene synthase 1 expression by cis-carotenoids", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 109, no. 46, 29 October 2012 (2012-10-29), pages 19021-19026, XP055314756, US ISSN: 0027-8424, DOI: 10.1073/pnas.1214808109	18-20
A	the whole document	1-17, 21-42

X	FRAY R G ET AL: "IDENTIFICATION AND GENETIC ANALYSIS OF NORMAL AND MUTANT PHYTOENE SYNTHASE GENES OF TOMATO BY SEQUENCING, COMPLEMENTATION AND CO-SUPPRESSION", PLANT MOLECULAR BIOLOGY, SPRINGER, DORDRECHT, NL, vol. 22, 1 January 1993 (1993-01-01), pages 589-602, XP002012920, ISSN: 0167-4412, DOI: 10.1007/BF00047400	18-20
A	the whole document	1-17, 21-42

X	YUAN D ET AL: "Genetics of flesh color and nucleotide sequence analysis of phytoene synthase gene 1 in a yellow-fruited tomato accession PI114490", SCIENTIA HORTICULTURAE, ELSEVIER SCIENCE PUBLISHERS, XX, vol. 118, no. 1, 2 September 2008 (2008-09-02), pages 20-24, XP023785679, ISSN: 0304-4238, DOI: 10.1016/J.SCIENTA.2008.05.011 [retrieved on 2008-06-26]	18-20
A	the whole document	1-17, 21-42

X	KANG BAOSHAN ET AL: "A chimeric transcript containingPsy1and a potential mRNA is associated withyellow fleshcolor in tomato accession PI 114490", PLANTA, SPRINGER VERLAG, DE, vol. 240, no. 5, 25 March 2014 (2014-03-25), pages 1011-1021, XP035408219, ISSN: 0032-0935, DOI: 10.1007/S00425-014-2052-Z [retrieved on 2014-03-25]	18-20
A	the whole document	1-17, 21-42

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