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(54) **BRASSICA OLERACEA PLANTS WITH IMPROVED NUTRITIONAL VALUE**

BRASSICA OLERACEA-PFLANZEN MIT VERBESSERTEM NÄHRWERT

PLANTS DE BRASSICA OLERACEA AVEC VALEUR NUTRITIONNELLE AMÉLIORÉE

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The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

Description

1. Field Of The Invention

[0001] The present invention relates to the development and use of *Brassica oleracea* plants with a recombined chromosomal segment.

2. Description of Related Art

[0002] Glucosinolates are allelochemicals present in 16 families of plant species, especially in *Brassicaceae*, of which broccoli is a notable example. Although there are over 120 different glucosinolates identified in nature, closely related taxonomic groups typically contain only a small number of such compounds. Glucosinolates that are dominant in broccoli such as glucoraphanin and glucoiberin are derived biochemically from the amino acid methionine. In the glucosinolate pathway, methionine is converted to homo-methionine and dihomomethionine by the activity of the ELONG (elongation) locus by adding a single carbon unit to the tail each time. Homo-methionine is eventually converted to 3-methylthiopropyl glucosinolate (glucoiberin; "MSP") while dihomomethionine is converted to 4-methylthiobutyl glucosinolate (glucoraphanin; "MSB"). These glucosinolates (glucoiberin and glucoraphanin) are potent inducers of phase II detoxification enzymes, such as glutathione-S-transferase and quinone reductase, which promote the metabolism and excretion of potential carcinogens.

SUMMARY OF THE INVENTION

[0003] In one aspect, the present invention provides a *Brassica oleracea* plant comprising a *Myb28* allele from *Brassica villosa* and lacking an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein the *Myb28* allele confers elevated glucosinolates when compared to a plant that lacks the *Myb28* allele.

[0004] In one embodiment, the plant is a broccoli plant. In other embodiments, the plant is inbred or hybrid. In another embodiment, the plant is homozygous for said *Myb28* allele from *Brassica villosa.* In yet another embodiment, the plant is heterozygous for said *Myb28* allele from *Brassica villosa.* In still another embodiment, the ELONG allele is from *Brassica oleracea.*

[0005] In another aspect, the present invention provides a part of a plant of the invention. In some embodiments, the plant part may further be defined as a leaf, an ovule, a floret, pollen, a head, or a cell.

[0006] In yet another aspect, the present invention provides a seed that produces a plant of the invention.

[0007] The present invention provides a plant as defined above and wherein a sample of seed comprising a chromosomal segment that comprises a *Myb28* allele from *Brassica villosa* and lacking an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein the segment confers elevated glucosinolates relative to a plant lacking the *Myb28* allele, was deposited under ATCC Accession Number PTA-13165. In one embodiment, the invention provides a seed that produces such a plant. In another embodiment, the invention provides a plant part, wherein the part is a leaf, an ovule, a floret, pollen, a head, or a cell. In another embodiment the plant is a *B. oleracea* plant.

[0008] We describe herein a recombined DNA segment comprising a *Myb28* allele from *Brassica villosa* and an ELONG allele from *Brassica oleracea* is provided. The DNA segment may be further defined as comprised within a cell. The DNA segment may be further defined as comprised within a seed. The DNA segment may be further defined as comprised within a plant.

[0009] Taught herein is a method for obtaining a *Brassica* plant comprising a desired glucosinolate composition comprising: a) obtaining a *Brassica* plant heterozygous for a *Myb28* allele from *Brassica villosa* that confers elevated glucosinolates and is genetically linked in the plant to an ELONG allele from *Brassica villosa;* (b) obtaining progeny of the plant; and (c) selecting at least a first progeny plant in which recombination has occurred such that the progeny comprises the *Myb28* allele but not the ELONG allele from *Brassica villosa*, wherein the progeny plant possesses a desired glucosinolate composition as a result of the presence of the *Myb28* allele but not the ELONG allele from *Brassica villosa.*

[0010] Selection of the progeny plant may comprise identifying a progeny plant that (1) comprises a genetic marker genetically linked to the *Myb28* allele in *Brassica villosa* and/or lacks a genetic marker present at the corresponding locus in said *Brassica* plant, and (2) lacks a genetic marker genetically linked to the ELONG allele from *Brassica villosa* and/or comprises a genetic marker present at the corresponding locus from said *Brassica* plant.

[0011] Selection of the progeny plant may comprise detecting a polymorphism that is found in the genome of said plant flanked by the complements of SEQ ID NO:1 and SEQ ID NO:2. Such allele(s) may be detected by a PCR-based method using oligonucleotide primer pair(s). Selection of the progeny plant may comprise detecting a polymorphism in said progeny plant that is shown in FIG. 5.

[0012] In yet a further aspect, the invention provides a plant produced by a method of the invention or a progeny thereof comprising the *Myb28* allele but not the ELONG allele from *Brassica villosa.* In one embodiment, the invention

provides a part of such a plant. In another embodiment, the part of the plant is selected from the group consisting of a cell, a seed, a root, a stem, a leaf, a head, a flower, and pollen.

[0013] Also taught herein is a method for producing a hybrid *Brassica oleracea* plant with elevated glucosinolate content comprising crossing a first *Brassica oleracea* parent plant with a second *Brassica oleracea* plant of a different genotype, wherein the first parent plant comprises a *Myb28* allele from *Brassica villosa* that lacks an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein the *Myb28* allele confers elevated glucosinolates relative to a plant lacking the *Myb28* allele. The method may further comprise producing a plurality of hybrid *Brassica oleracea* plants comprising crossing the first *Brassica oleracea* parent plant with a plurality of second *Brassica oleracea* plants of different genotypes.

[0014] We describe herein a method of producing a *Brassica oleracea* plant with a desired elevated glucosinolate content comprising introgressing into the plant a chromosomal segment comprising a *Myb28* allele from *Brassica villosa* and lacking an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein segment confers a desired glucosinolate content relative to a plant lacking the segment, wherein a sample of seed comprising the chromosomal segment is deposited under ATCC Accession No. PTA-13165.

[0015] The term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and to "and/or." When used in conjunction with the word "comprising" or other open language in the claims, the words "a" and "an" denote "one or more," unless specifically noted. The terms "comprise," "have" and "include" are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as "comprises," "comprising," "has," "having," "includes" and "including," are also open-ended. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps. Similarly, any plant that "comprises," "has" or "includes" one or more traits is not limited to possessing only those one or more traits and covers other unlisted traits.

[0016] Other objects, features and advantages of the present invention will become apparent from the following detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017]

FIG. 1 depicts the head from hybrid broccoli varieties Ironman (left) and RX05991199 (right). Varieties were grown in Summer at a spacing of 50x50cm.

FIG. 2 shows a horizontal cross section of stems from hybrid broccoli varieties Ironman (left) and RX05991199 (right). Varieties were grown in Autumn at a spacing of 50x50cm.

FIG. 3 shows a profile of the head and stem from hybrid broccoli varieties Ironman (left) and RX05991199 (right). Varieties were grown in Autumn at a spacing of 50x50cm.

FIG. 4 shows assay results for different ELONG alleles with the QTL1-BoGLS-ELONG marker. The V allele is the *B. villosa* allele associated with Myb28, which results in high 3-MSP/4-MSB ratio and high total glucosinolates. The A, B and C alleles are examples of alleles found in broccoli without a *B. villosa* ELONG allele.

FIG. 5 shows an alignment between a consensus sequence of the *Myb28* locus from *B. villosa* contained in broccoli variety FT69, and a consensus sequence of the corresponding locus from broccoli without increased level of glucosinolate, *e.g. B. oleracea,* (Oleracea) (SEQ ID NOs.:8-9).

DETAILED DESCRIPTION OF THE INVENTION

[0018] The invention provides methods and plants, seeds and derivatives of *Brassica oleracea* plants comprising a new recombined introgression from *Brassica villosa* capable of conferring elevated 4-methylsulfinylbutyl glucosinolates (MSB), also known as glucoraphanin. It was also surprisingly found that plants comprising the introgression were capable of consistently producing hybrids that exhibited elevated glucoraphanin content relative to glucoiberin, while results were substantially more variable when using a parent line comprising the non-recombined introgression, such as in the case of the Myb28 donor parent of the hybrid PS05151639, which comprises Myb28 and ELONG alleles from *Brassica villosa* (US Patent Appln Pub No. 2011/0055945). The ability to produce multiple elite hybrid progeny with elevated glucoraphanin content and/or ratio of glucoraphanin to glucoiberin from a single inbred parent is significant in that the number of elite

Brassica oleracea parent lines available to produce hybrid varieties is limited. The reduced introgression therefore substantially increases the utility of a given inbred and allows, for example, the production of multiple hybrids potentially adapted to different growing environments, end uses, or other criteria, each having a desired glucoraphanin content.

[0019] The new "reduced introgression" comprising a *Myb28* locus from *Brassica villosa* and lacking the ELONG locus of *Brassica villosa* that has to date been genetically linked thereto is capable of consistently conferring in hybrids derived from a plant comprising the introgression elevated glucoraphanin relative to glucoiberin. One aspect of the current invention thus concerns methods for obtaining a *Brassica oleracea* plant comprising at least one such reduced introgression, wherein the resulting *Brassica oleracea* plant and/or progeny derived therefrom displays a desired glucoraphanin content relative to a control plant lacking the introgression. The invention thus provides plants possessing a desired glucoraphanin content conferred by a reduced introgression of the invention. In certain embodiments, methods for obtaining such plants comprise obtaining a *Brassica oleracea* plant heterozygous for a *Myb28* allele from *Brassica villosa* that confers elevated glucosinolates and is genetically linked in the plant to an ELONG allele from *Brassica villosa*, obtaining progeny from such a plant, and selecting one or more such progeny plants wherein genetic recombination has occurred such that the progeny comprises an *Myb28* allele from *Brassica villosa*, but does not comprise an ELONG allele from *Brassica villosa.* Such progeny or further progeny thereof may also possess a desired glucoraphanin content as a result of the presence of the *Myb28* allele but not the ELONG allele from *Brassica villosa.* In particular embodiments, the method may comprise obtaining a progeny plant that comprises such allele(s) by identifying one or more genetic markers genetically linked to the *Myb28* and/or ELONG allele(s). Identifying the genetic markers may comprise a phenotypic, a genetic, or a biochemical test, and may include screening a parent and/or progeny plant for the presence of, for instance, one or more allele described herein, including, for example, a *Myb28* allele from *B. villosa*, an ELONG allele from *B. villosa* and an ELONG allele from *B. oleracea.*

[0020] Certain traits such as a glucoraphanin content relative to glucoiberin content, or the unpredictability of the type of glucosinolates in hybrid progeny heterozygous for a *Brassica villosa Myb28* allele, were found to co-locate with a glucosinolate trait in the *Myb28* allele from *Brassica villosa* and ELONG allele from *Brassica villosa* introgressions. Thus, formation of a "reduced" introgression is understood to be caused by recombination event(s) in the vicinity of the *Myb28* and ELONG QTL(s). Lines comprising a reduced introgression, i.e., which have undergone a recombination event close to the QTL having elevated glucosinolates may efficiently be screened by use of molecular and/or phenotypic markers. Thus, plant populations or progeny of such populations, segregating (i.e., heterozygous) with respect to the QTL specified by *Myb28* and ELONG introgressions, may be screened for plants having a recombinant phenotype, *e.g*. elevated glucoraphanin levels relative to glucoiberin levels.

[0021] In other embodiments, a method of the invention may comprise identifying a *Brassica oleracea* plant comprising a *Brassica villosa*-derived reduced introgression, and comprising a meiotic recombination between *Myb28* and ELONG alleles as described herein. In particular embodiments, identifying the introgression may comprise measuring glucoraphanin and/or glucoiberin using standard protocols. In certain embodiments, a plant of the invention comprising a reduced introgression as disclosed herein comprises an elevated average proportion of glucoraphanin relative to glucoiberin compared to a plant comprising *Myb28* and ELONG alleles from *Brassica villosa*, or a plant lacking a *Myb28* allele from *Brassica villosa.* In one embodiment, such a plant comprising an elevated average proportion of glucoraphanin relative to glucoiberin is an inbred line, and in another embodiment is defined as a F1 hybrid having as one or more parent a plant comprising a reduced introgression of the invention. In particular embodiments, a plant of the invention is provided comprising a ratio of glucoraphanin to glucoiberin of about 10:1, 12:1, 15:1, 18:1, 20:1, 23:1, 25:1, 28:1, 30:1, 35:1 and about 40:1. In one aspect, an increase in glucoraphanin content may be calculated in reference to a standard *Brassica oleracea* variety, such as the broccoli variety Ironman.

A. Breeding of ***Brassica oleracea*** **Lines Displaying Elevated Glucoraphanin**

[0022] We describe herein methods for crossing a plant comprising a reduced *Myb28*/ELONG introgression provided herein with itself or a second plant and the seeds and plants produced by such methods. These methods can be used for production and propagation of cultivated *Brassica oleracea* plants displaying desired glucosinolate compositions, including MSB and/or MSP content. Yet further, the plants of the current invention having elevated glucosinolate content comprise improved nutritional value of the plant relative to plants without elevated glucosinolates. The methods also can be used to produce hybrid *Brassica oleracea* seeds and the plants grown therefrom. Hybrid seeds are produced by crossing such lines with a second *Brassica oleracea* parent line. The hybrids may be heterozygous or homozygous for the reduced introgression.

[0023] *Brassica villosa* is a wild species endemic to northwest and central Sicily, and thus a Myb28 allele could be obtained by one of skill in the art from a plant selected from the wild. Alternatively, Myb28 alleles are known in the art and may be obtained from other sources for use with the invention, including SNR 347 (FT69; referred to as 428-11-69 in Mithen et al., Theor Appl Genet, 106:727-734; 2003), BR384-014, SNP13 (580333), SNP88 (BRM51-1210), BR384-020, B1639 (ATCC Accession Number PTA-9676), BRM51-1162 (ATCC Accession Number PTA-9675) and RX

05991199 (ATCC Accession No. PTA-13165). In accordance with the invention, a plant provided herein will generally lack an ELONG allele from *Brassica villosa* genetically linked to the *Myb28* allele. This can be achieved according to the invention through crossing a plant comprising *Myb28* and ELONG alleles from *Brassica villosa* with *Brassica* plants not comprising the *Myb28* and ELONG alleles from *Brassica villosa,* including standard *Brassica oleracea* varieties. This includes the many broccoli varieties well known in the art, among others.

[0024] The goal of vegetable breeding is to combine various desirable traits in a single variety/hybrid. Such desirable traits may include any trait deemed beneficial by a grower and/or consumer, including greater yield, resistance to insects or disease, tolerance to environmental stress, and nutritional value. Breeding techniques used in an attempt to obtain desired traits take advantage of a plant's method of pollination. There are two general methods of pollination: a plant self-pollinates if pollen from one flower is transferred to the same or another flower of the same plant. A plant cross-pollinates if pollen comes to it from a flower of a different plant.

[0025] The development of uniform varieties requires the development of homozygous inbred plants, the crossing of these inbred plants, and the evaluation of the crosses. Pedigree breeding and recurrent selection are examples of breeding methods that have been used to develop inbred plants from breeding populations. Those breeding methods combine the genetic backgrounds from two or more plants or various other broad-based sources into breeding pools from which new lines and hybrids derived therefrom are developed by selfing and selection of desired phenotypes.

[0026] Novel varieties may be created by crossing plants of the invention followed by generations of selection as desired and inbreeding for development of uniform lines. New varieties may also be created by crossing with any second plant. In selecting such a second plant to cross for the purpose of developing novel lines, it may be desired to choose those plants which either themselves exhibit one or more selected desirable characteristics or which exhibit the desired characteristic(s) when in hybrid combination. Once initial crosses have been made, inbreeding and selection take place to produce new varieties. For development of a uniform line, often five or more generations of selfing and selection are typically involved.

[0027] Uniform lines of new varieties may also be developed by way of doubled-haploids. This technique allows the creation of true breeding lines without the need for multiple generations of selfing and selection. In this manner true breeding lines can be produced in as little as one generation. Haploid embryos may be produced from microspores, pollen, anther cultures, or ovary cultures. The haploid embryos may then be doubled autonomously, or by chemical treatments (*e.g*. colchicine treatment). Alternatively, haploid embryos may be grown into haploid plants and treated to induce chromosome doubling. In either case, fertile homozygous plants are obtained. In accordance with the invention, any of such techniques may be used in connection with a plant of the present invention and progeny thereof to achieve a homozygous line.

[0028] Backcrossing can also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait, such as elevated glucoraphanin, from one inbred or non-inbred source to a variety that lacks that trait. This can be accomplished, for example, by first crossing a parent (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate locus or loci for the trait in question. The progeny of this cross are then mated back to the recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the first parent for most or almost all other loci. The last backcross generation would be selfed to give pure breeding progeny for the trait being transferred.

[0029] The selection of a suitable recurrent parent is an important step for a successful backcrossing procedure. The goal of a backcross protocol is to alter or substitute a single trait or characteristic in the original variety. To accomplish this, a single locus of the recurrent variety is modified or substituted with the desired locus from the nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent will depend on the purpose of the backcross; one of the major purposes is to add some commercially desirable trait to the plant. The exact backcrossing protocol will depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele may also be transferred. It may be necessary to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

[0030] *Brassica oleracea* varieties can also be developed from more than two parents. The technique, known as modified backcrossing, uses different recurrent parents during the backcrossing. Modified backcrossing may be used to replace the original recurrent parent with a variety having certain more desirable characteristics or multiple parents may be used to obtain different desirable characteristics from each.

[0031] Many single locus traits have been identified that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits may or may not be transgenic; examples of these traits include, but are not limited to, male sterility, herbicide resistance, resistance to bacterial, fungal, or viral disease, insect resistance, restoration of male fertility, modified fatty acid or carbohydrate metabolism, and enhanced nutritional quality. These comprise genes generally inherited through the nucleus.

[0032] Direct selection may be applied where the single locus acts as a dominant trait. Selection of *Brassica* plants for breeding is not necessarily dependent on the phenotype of a plant and instead can be based on genetic investigations. For example, one can utilize a suitable genetic marker which is closely genetically linked to a trait of interest. One of these markers can be used to identify the presence or absence of a trait in the offspring of a particular cross, and can be used in selection of progeny for continued breeding. This technique is commonly referred to as marker assisted selection. Any other type of genetic marker or other assay which is able to identify the relative presence or absence of a trait of interest in a plant can also be useful for breeding purposes.

[0033] Procedures for marker assisted selection are of particular utility for introgression of given traits. Well known types of genetic markers that could be used in accordance with the invention include, but are not necessarily limited to, Simple Sequence Length Polymorphisms (SSLPs), Cleaved Amplified Polymorphic Sequences (CAPs), Randomly Amplified Polymorphic DNAs (RAPDs), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Arbitrary Primed Polymerase Chain Reaction (AP-PCR), Amplified Fragment Length Polymorphisms (AFLPs), and Single Nucleotide Polymorphisms (SNPs).

B. Plants Derived From a Plant of the Invention by Genetic Engineering

[0034] Many useful traits that can be introduced by backcrossing, as well as directly into a plant, are those, which may be introduced by genetic transformation techniques. Genetic transformation may therefore be used to insert a selected transgene into a *Brassica* plant of the invention or may, alternatively, be used for the preparation of transgenes, which can be introduced by backcrossing. Methods for the transformation of plants, including *Brassica,* are well known to those of skill in the art.

[0035] Vectors used for the transformation of plant cells are not limited so long as the vector can express an inserted DNA in the cells. For example, vectors comprising promoters for constitutive gene expression in *Brassica* cells (*e.g.*, cauliflower mosaic virus 35S promoter) and promoters inducible by exogenous stimuli can be used. Examples of suitable vectors include pBI binary vector. The "*Brassica* cell" into which the vector is to be introduced includes various forms of *Brassica* cells, such as cultured cell suspensions, protoplasts, leaf sections, and callus.

[0036] A vector can be introduced into *Brassica* cells by known methods, such as the polyethylene glycol method, polycation method, electroporation, *Agrobacterium*-mediated transfer, particle bombardment and direct DNA uptake by protoplasts.

[0037] To effect transformation by electroporation, one may employ either friable tissues, such as a suspension culture of cells or embryogenic callus or alternatively one may transform immature embryos or other organized tissue directly. In this technique, one would partially degrade the cell walls of the chosen cells by exposing them to pectin-degrading enzymes (pectolyases) or mechanically wound tissues in a controlled manner.

[0038] One efficient method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, particles are coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, platinum, and preferably, gold. For the bombardment, cells in suspension are concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded can be positioned at an appropriate distance below the macroprojectile stopping plate. Microprojectile bombardment techniques are widely applicable, and may be used to transform virtually any plant species.

[0039] *Agrobacterium*-mediated transfer is another widely applicable system for introducing gene loci into plant cells. An advantage of the technique is that DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium* (and other Rhizobia), allowing for convenient manipulations. Moreover, recent technological advances in vectors for *Agrobacterium-mediated* gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate the construction of vectors capable of expressing various polypeptide coding genes. The vectors described have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes. Additionally, *Agrobacterium* containing both armed and disarmed Ti genes can be used for transformation.

[0040] In those plant strains where *Agrobacterium-mediated* transformation is efficient, it is the method of choice because of the facile and defined nature of the gene locus transfer. The use of *Agrobacterium-mediated* plant integrating vectors to introduce DNA into plant cells is well known in the art (U.S. Patent No. 5,563,055). For example, U.S. Patent No. 5,349,124 describes a method of transforming plant cells using *Agrobacterium-mediated* transformation. By inserting a chimeric gene having a DNA coding sequence encoding for the full-length B.t. toxin protein that expresses a protein toxic toward Lepidopteran larvae, this methodology resulted in plants having resistance to such insects.

[0041] A number of promoters have utility for plant gene expression for any gene of interest including but not limited to selectable markers, scorable markers, genes for pest tolerance, disease resistance, nutritional enhancements and any other gene of agronomic interest. Examples of constitutive promoters useful for *Brassica* plant gene expression

include, but are not limited to, the cauliflower mosaic virus (CaMV) P-35S promoter, which confers constitutive, high-level expression in most plant tissues, including monocots; a tandemly duplicated version of the CaMV 35S promoter, the enhanced 35S promoter (P-e35S) the nopaline synthase promoter, the octopine synthase promoter; and the figwort mosaic virus (P-FMV) promoter as described in U.S. Pat. No. 5,378,619 and an enhanced version of the FMV promoter (P-eFMV) where the promoter sequence of P-FMV is duplicated in tandem, the cauliflower mosaic virus 19S promoter, a sugarcane bacilliform virus promoter, a commelina yellow mottle virus promoter, and other plant DNA virus promoters known to express in plant cells.

[0042] Exemplary nucleic acids which may be introduced to the plants of this invention include, for example, DNA sequences or genes from another species, or even genes or sequences which originate with or are present in the same species, but are incorporated into recipient cells by genetic engineering methods rather than classical reproduction or breeding techniques. However, the term "exogenous" is also intended to refer to genes that are not normally present in the cell being transformed, or perhaps simply not present in the form, structure, *etc.,* as found in the transforming DNA segment or gene, or genes which are normally present and that one desires to express in a manner that differs from the natural expression pattern, *e.g.*, to over-express. Thus, the term "exogenous" gene or DNA is intended to refer to any gene or DNA segment that is introduced into a recipient cell, regardless of whether a similar gene may already be present in such a cell. The type of DNA included in the exogenous DNA can include DNA which is already present in the plant cell, DNA from another plant, DNA from a different organism, or a DNA generated externally, such as a DNA sequence containing an antisense message of a gene, or a DNA sequence encoding a synthetic or modified version of a gene.

[0043] Many hundreds if not thousands of different genes are known and could potentially be introduced into a *Brassica* plant according to the invention. Non-limiting examples of particular genes and corresponding phenotypes one may choose to introduce into a *Brassica* plant include one or more genes for insect tolerance, such as a *Bacillus thuringiensis* (B.t.) gene, pest tolerance such as genes for fungal disease control, herbicide tolerance such as genes conferring glyphosate tolerance, and genes for quality improvements such as yield, nutritional enhancements, environmental or stress tolerances, or any desirable changes in plant physiology, growth, development, morphology or plant product(s). For example, structural genes would include any gene that confers insect tolerance including but not limited to a *Bacillus* insect control protein gene as described in WO 99/31248, U.S. Pat. No. 5,689,052, U.S. Pat. Nos. 5,500,365 and 5,880,275. In another embodiment, the structural gene can confer tolerance to the herbicide glyphosate as conferred by genes including, but not limited to *Agrobacterium* strain CP4 glyphosate resistant EPSPS gene (aroA:CP4) as described in U.S. Pat. No. 5,633,435, or glyphosate oxidoreductase gene (GOX) as described in U.S. Pat. No. 5,463,175.

[0044] Alternatively, the DNA coding sequences can affect these phenotypes by encoding a non-translatable RNA molecule that causes the targeted inhibition of expression of an endogenous gene, for example via antisense- or co-suppression-mediated mechanisms. The RNA could also be a catalytic RNA molecule (*i.e.*, a ribozyme) engineered to cleave a desired endogenous mRNA product. Thus, any gene which produces a protein or mRNA which expresses a phenotype or morphology change of interest is useful for the practice of the present invention.

D. Definitions

[0045] In the description and tables herein, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided:

Allele: Any of one or more alternative forms of a gene locus, all of which alleles relate to one trait or characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

Backcrossing: A process in which a breeder repeatedly crosses hybrid progeny, for example a first generation hybrid (F_1), back to one of the parents of the hybrid progeny. Backcrossing can be used to introduce one or more single locus conversions from one genetic background into another.

Cultivated Variety: A *Brassica oleracea* variety which is suitable for consumption and meets the requirements for commercial cultivation. An example is a broccoli variety. In addition to the plants themselves, and the parts thereof suitable for consumption, such as the heads or leaves, the invention comprises parts or derivatives of the plant suitable for propagation. Examples of parts suitable for propagation are organ tissues, such as leaves, stems, roots, shoots and the like, protoplasts, somatic embryos, anthers, petioles, cells in culture and the like. Derivatives suitable for propagation are for instance seeds. The plants according to the invention can be cultivated or propagated in the conventional manner but also by means of tissue culture techniques from plant parts.

Crossing: The mating of two parent plants.

Cross-pollination: Fertilization by the union of two gametes from different plants.

Diploid: A cell or organism having two sets of chromosomes.

Enzymes: Molecules which can act as catalysts in biological reactions.

F_1 Hybrid: The first generation progeny of the cross of two nonisogenic plants.

Genotype: The genetic constitution of a cell or organism.
Haploid: A cell or organism having one set of the two sets of chromosomes in a diploid.
Linkage: A phenomenon wherein alleles on the same chromosome tend to segregate together more often than expected by chance if their transmission was independent.
Marker: A readily detectable phenotype, preferably inherited in co-dominant fashion (both alleles at a locus in a diploid heterozygote are readily detectable), with no environmental variance component, *i.e.*, heritability of 1.
Phenotype: The detectable characteristics of a cell or organism, which characteristics are the manifestation of gene expression.
Quantitative Trait Loci (QTL): Quantitative trait loci (QTL) refer to genetic loci that control to some degree numerically representable traits that are usually continuously distributed.
Recombination event is understood to mean a meiotic crossing-over.
Regeneration: The development of a plant from tissue culture.
Self-pollination: The transfer of pollen from the anther to the stigma of the same plant.
Single Locus Converted (Conversion) Plant: Plants which are developed by a plant breeding technique called backcrossing, wherein essentially all of the desired morphological and physiological characteristics of a broccoli variety are recovered in addition to the characteristics of the single locus transferred into the variety *via* the backcrossing technique and/or by genetic transformation.
Substantially Equivalent: A characteristic that, when compared, does not show a statistically significant difference (*e.g.*, p = 0.05) from the mean.
Tissue Culture: A composition comprising isolated cells of the same or a different type or a collection of such cells organized into parts of a plant.
Transgene: A genetic locus comprising a sequence which has been introduced into the genome of a broccoli plant by transformation.

[0046] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the invention, as limited only by the scope of the appended claims.

E. Deposit Information

[0047] A deposit was made of at least 2500 seeds of broccoli hybrid RX 05991199, which comprises a reduced introgression comprising a *Myb28* allele from *Brassica villosa* and an ELONG allele from *Brassica oleracea,* as described herein. The deposit was made with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209 USA. The deposit is assigned ATCC Accession No. PTA-13165. The date of deposit was August 24, 2012. Access to the deposits will be available during the pendency of the application to persons entitled thereto upon request. The deposits will be maintained in the ATCC Depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent, whichever is longer, and will be replaced if nonviable during that period. Applicant does not waive any infringement of their rights granted under this patent or any other form of variety protection, including the Plant Variety Protection Act (7 U.S.C. 2321 *et seq.*).

EXAMPLES

Example 1

Development of Parent Lines With Improved MSB Profiles

[0048] One benefit of the current invention is that parent lines may be created comprising a reduced introgression of the invention, wherein the lines and hybrids derived therefrom exhibit an increased proportion of MSB/MSP and/or a greater stability of expression of MSB. The development of such a broccoli line comprising a Myb28/ELONG reduced introgression can be summarized as follows:
[0049] The line FT69, which has elevated levels of the phytochemical MSP (glucoiberin) as a result of the presence of *Myb28* and ELONG loci from *B. villosa* (Mithen et al., 2003; Theor. Appl. Genet., 106:727-734), was crossed with a breeding line designated BR9. The resulting progeny was crossed with the male parent from Ironman (BRM 56-3905 SI). F1 progeny were grown in replications in a Wageningen selection trial. From plots designated 408, 409, 410, 411 and 412, 89 plants were selected that were analyzed for glucoiberin (MSP) and glucoraphanin (MSB). Six plants with the highest MSB were selected.
[0050] All 6 plants were selfed and backcrossed with the recurrent parent BRM 56-3905 SI. The 6 selfings and 6 BC1 were planted in replicated selection trials in Wageningen. Each BC1 (where BC = backcross) had 2 plots with 24

plants/plot. From these 12 plots, a total of 84 plants were selected that most resembled the recurrent parent. Heads from these selections were sent for MSP and MSB analyses. Seven plants with highest MSB were kept for further selfing and BC. All 7 BC1 plants were selfed and backcrossed with the recurrent parent (BRM 56-3905 SI). Selfings and 5 BC2 populations were sown and selected in replicated Wageningen Autumn selection trials. A total of 73 plants that resembled the recurrent parent most were selected and sent for MSP/MSB analyses. Eight BC2 plants with highest MSB levels were kept for further selfing and BC.

[0051] All 8 BC2 plants were selfed and backcrossed with the recurrent parent (BRM 56-3905 SI). Four BC3 were obtained and planted in 1-4 replications in Wageningen. 47 plants closest to the recurrent parent were sent for MSP/MSB analyses. 7 BC3 plants with highest MSB levels were kept for further selfing and BC. All 7 BC3 plants were selfed and backcrossed with the recurrent parent (BRM 56-3905 SI). 6 BC4 were obtained and these were planted in selection trials. 54 plants closest to the recurrent parent were sent for MSP/MSB analyses. 8 BC4 plants with the highest MSB levels were kept for further selfing and BC.

[0052] All 8 BC4 plants were selfed. 7 BC4 plants produced selfed seed and the BC4F2 were planted in unreplicated Wageningen selection trials. 37 BC4F2 plants closest to the recurrent parent were selected and these were sent for MSP/MSB analyses. 4 BC4F2 plants with highest MSB levels were kept for further selfing and BC.

[0053] All 4 BC4F2 plants were selfed. All 4 produced selfed seed and the 4 BC4F3 were planted in unreplicated selection trials. 12 BC4F3 plants closest to the recurrent parent were selected and sent for MSP/MSB analyses. 4 BC4F2 plants with the highest MSB levels were kept for further selfing and BC. All selected plants from plot 1169 (570114) had equally high MSB level of average 2.3 mmol/kg, or about 5x the level of the standard reference variety (General). This indicated that the source has alleles fixed for high MSB.

[0054] All 3 selections from 1169 (BC4F3) were selfed. All 3 produced seed and these were planted in selection trials. SNP13-580333 (1169-1, A75-1) was the most uniform on type and 5 plants were selected for MSB check. All 5 had equally high levels of MSB of about 3.2 mmol/kg. 1169-1, also designated BRM 53-3934 SI, was put on tissue culture for increase and use as a male parent to RX-1199 (RX 05991199). An analysis of BRM 53-3934 SI revealed that it contained the *Myb28* reduced introgression, which results in a parent line with the ability to consistently produce progeny with glucosinolates comprising an increased proportion of MSB/MSP relative to plants having the *B. villosa* ELONG locus, as shown below.

Example 2

Analysis of Hybrid Broccoli Lines Having Elevated Glucoraphanin

[0055] Self and outcrosses were made using different standard broccoli lines as female parents as shown in Table 1 in crosses with the following: (1) FT69 (high glucoiberin line with ELONG and Myb28 from *B. villosa*), (2) SNP13-580333 (high glucoraphanin line with ELONG from *B. oleracea* and Myb28 from *B. villosa*, as described above), and (3) SNP88-BRM51-1210 (high glucoiberin line ELONG and Myb28 from *B. villosa*). As can be seen, regular broccoli lines have a relatively low amount of total glucosinolates. The ratio between MSB and MSP is particularly shown to be depending on the regular broccoli female line in the case of FT69 (428-11-69), which comprises ELONG and Myb28 alleles from *B. villosa.* In contrast, in all the crosses with SNP13-580333 most of the glucosinolates are glucoraphanin. This indicates the benefit of the reduced introgression in consistently producing hybrid varieties comprising elevated glucoraphanin in crosses with multiple different second parents.

Table 1: Levels of Glucoraphanin Resulting From Different Crosses

Female line (regular broccoli line)	Male self (self or crosses with different high glucosinolates sources)	Glucoiberin micromoles /gramDWt	Glucoraphanin micromoles /gramDWt	Ratio MSB/MSP
BR384-014	self	3.31	18.74	5.66
BR384-014	FT69 (428-11-69) (contains Myb28 and ELONG from B. *villosa*)	57.47	15.12	0.26
BR384-014	SNP13-580333 (BRM 53-3934 SI) (contains reduced introgression)	3.77	40.14	10.65
BR384-014	SNP88-BRM51-1210 (contains Myb28 and ELONG from *B. villosa*)	28.71	5.85	0.20
BR384-020	Self	1.83	18.24	9.97

(continued)

Female line (regular broccoli line)	Male self (self or crosses with different high glucosinolates sources)	Glucoiberin micromoles /gramDWt	Glucoraphanin micromoles /gramDWt	Ratio MSB/MSP
BR384-020	FT69 (428-11-69)	36.72	10.00	0.27
BR384-020	SNP13-580333	2.34	26.29	11.23
BR384-020	SNP88-BRM51-1210	13.53	33.96	2.51
BR384-024	self	1.59	3.64	2.29
BR384-024	FT69 (428-11-69)	56.61	7.75	0.14
BR384-024	SNP13-580333	2.64	26.48	10.03
BR384-024	SNP88-BRM51-1210	22.11	3.24	0.15
BR384-025	self	1.43	2.67	1.87
BR384-025	FT69 (428-11-69)	39.66	6.39	0.16
BR384-025	SNP13-580333	3.52	27.55	7.83
BR384-025	SNP88-BRM51-1210	14.25	9.59	0.67
BR384-026	self	1.74	5.08	2.92
BR384-026	FT69 (428-11-69)	41.11	8.26	0.20
BR384-026	SNP13-580333	1.80	30.53	16.93
BR384-026	SNP88-BRM51-1210	19.52	2.89	0.15
BR384-027	self	2.06	13.52	6.55
BR384-027	FT69 (428-11-69)	9.32	47.34	5.08
BR384-027	SNP13-580333	2.60	27.47	10.57
BR384-027	SNP88-BRM51-1210	6.61	28.12	4.26
BR384-033	self	0.57	16.88	29.42
BR384-033	FT69 (428-11-69)	7.58	46.89	6.18
BR384-033	SNP13-580333	2.48	30.81	12.41
BR384-033	SNP88-BRM51-1210	5.23	28.75	5.49
BR384-040	self	0.91	14.03	15.46
BR384-040	FT69 (428-11-69)	4.77	45.61	9.56
BR384-040	SNP13-580333	1.56	16.02	10.27
BR384-040	SNP88-BRM51-1210	3.30	12.73	3.86
BR384-044	self	0.81	11.94	14.74
BR384-044	FT69 (428-11-69)	9.48	50.08	5.28
BR384-044	SNP13-580333	1.99	23.47	11.81
BR384-044	SNP88-BRM51-1210	5.19	27.95	5.38
BR384-048	FT69 (428-11-69)	62.53	5.85	0.09
BR384-048	SNP13-580333	3.02	27.95	9.25
BR384-048	SNP88-BRM51-1210	18.99	1.24	0.07
BR384-052	self	1.51	7.38	4.88
BR384-052	FT69 (428-11-69)	8.27	38.54	4.66

(continued)

Female line (regular broccoli line)	Male self (self or crosses with different high glucosinolates sources)	Glucoiberin micromoles /gramDWt	Glucoraphanin micromoles /gramDWt	Ratio MSB/MSP
BR384-052	SNP13-580333	2.43	24.09	9.92
BR384-053	self	0.28	2.06	7.49
BR384-053	FT69 (428-11-69)	7.30	53.95	7.39
BR384-053	SNP13-580333	0.64	21.64	33.74
BR384-053	SNP88-BRM51-1210	3.39	14.50	4.27
BR384-057	self	1.43	3.79	2.64
BR384-057	FT69 (428-11-69)	38.32	8.11	0.21
BR384-057	SNP13-580333	4.13	32.20	7.80
BR384-057	SNP88-BRM51-1210	20.38	3.47	0.17
BR384-060	self	1.76	7.90	4.49
BR384-060	FT69 (428-11-69)	10.62	42.68	4.02
BR384-060	SNP13-580333	2.76	26.06	9.46
BR384-060	SNP88-BRM51-1210	5.46	23.19	4.25
BR384-061	self	0.99	15.47	15.65
BR384-061	FT69 (428-11-69)	8.03	49.14	6.12
BR384-061	SNP13-580333	2.58	35.61	13.82
BR384-061	SNP88-BRM51-1210	5.92	36.02	6.09
BR384-069	self	1.63	7.02	4.32
BR384-069	FT69 (428-11-69)	9.33	56.09	6.01
BR384-069	SNP13-580333	1.44	26.36	18.32
BR384-069	SNP88-BRM51-1210	4.60	29.96	6.51
BR384-078	self	1.23	7.59	6.15
BR384-078	FT69 (428-11-69)	10.54	36.99	3.51
BR384-078	SNP13-580333	2.75	24.94	9.06
BR384-078	SNP88-BRM51-1210	5.74	23.42	4.08
BR384-082	self	1.57	10.41	6.64
BR384-082	FT69 (428-11-69)	10.67	38.80	3.64
BR384-082	SNP13-580333	2.53	29.25	11.55
BR384-082	SNP88-BRM51-1210	6.38	31.80	4.98
BR384-083	self	1.31	8.44	6.45
BR384-083	FT69 (428-11-69)	9.33	39.32	4.21
BR384-083	SNP13-580333	2.23	22.39	10.04
BR384-083	SNP88-BRM51-1210	4.85	21.90	4.52
BR384-089	self	3.40	0.35	0.10
BR384-089	FT69 (428-11-69)	48.47	7.21	0.15
BR384-089	SNP13-580333	4.67	24.59	5.27

(continued)

Female line (regular broccoli line)	Male self (self or crosses with different high glucosinolates sources)	Glucoiberin micromoles /gramDWt	Glucoraphanin micromoles /gramDWt	Ratio MSB/MSP
BR384-089	SNP88-BRM51-1210	19.12	1.98	0.10

EXAMPLE 3

Development of Broccoli Hybrids

[0056] As explained above, one embodiment of the invention comprises producing broccoli hybrids wherein one or both parents of the hybrid comprise a Myb28 reduced introgression and, as a result, exhibit an elevated MSB content and/or more stable MSB content relative to hybrids lacking a parent comprising the introgression. One example of such a hybrid that was produced is the hybrid RX 05991199. This hybrid was created by crossing parents BRM 53-3934 SI and BRM 56-3907 CMS, typically with BRM 53-3934 SI as a male parent. The production of BRM 53-3934 SI is described in Example 1 above. As explained, this parent contains the *Myb28* reduced introgression. The female parent BRM 56-3907 CMS is a known inbred that served as the parent of the commercial hybrid "Ironman". BRM 56-3907 CMS is the subject of, and is described in, EU Plant Variety Rights Certificate #20341, granted June 18, 2007.

[0057] A description of the physiological and morphological characteristics of broccoli hybrid RX 05991199 and the parent lines thereof is presented below.

Table 2: Physiological and Morphological Characteristics of Hybrid RX 05991199

	Characteristic	RX05991199CMS	Ironman
A	Region of Adaptation	NW Europe	NW Europe
B	Maturity, Spring Planted		
	days from direct seeding to 50% harvest	no direct seeding	no direct seeding
	days from transplanting to 50% harvest	64	63
	transplant date	10-May-10	10-May-10
	length of harvest period in days	8	6
	first harvest date	12-Jul- 10	12-Jul-10
	last harvest date	19-Jul-10	17-Jul-10
	harvest season (main crop at 50% harvest)	summer	summer
	time of harvest maturity (50% of plants)	medium (Sumosun)	medium (Sumosun)
	time of beginning of flowering (50% of plants with at least 10% flowers) **choice for UPOV TG only	medium*8 (Coaster, Cruiser)	medium*8 (Coaster, Cruiser)
C	Seedling		
	cotyledon color	medium green	medium green
	RHS color chart value for seedling cotyledon color	147B	147B
	cotyledon anthocyanin	intermediate	intermediate
	hypocotyl anthocyanin	strong	strong

(continued)

D	Plant		
	plant height in centimeters from soil line to top of leaves	56.3 cm	61.0 cm
	head height in centimeters from soil line to top of leaves	33.7 cm	34.7 cm
	height at harvest maturity	medium (Coaster)	medium (Coaster)
	number of stems	one (Ramoso Calabrese, Shogun)	one (Ramoso Calabrese, Shogun)
	branches	medium	few
	habit	intermediate	intermediate
	market class	fresh market	fresh market
	life cycle	annual	annual
	type of variety	first generation hybrid	first generation hybrid
E	Leaves		
	outer leaves: number of leaves per plant (at harvest)	14	17
	outer leaves: width (at midpoint of plant including petiole)	22.6 cm	24.2 cm
	leaf: width	medium (Buccaneer, Green Belt)	medium (Buccaneer, Green Belt)
	outer leaves: length (at midpoint of plant including petiole)	48.7 cm	53.2 cm
	Characteristic	**RX05991199CMS**	**Ironman**
	leaf: length (including petiole)	medium (Brigadeer, Sumosun)	long (Green Duke, Laser)
	outer leaves: petiole length	22.1 cm	24.9 cm
	petiole: length	medium (Emperor, Ramoso Calabrese)	long (Groene Calabrese, Premium Crop)
	outer leaves: leaf ratio - length/ width	2:1	2:1
	outer leaves: leaf attachment	petiolate	petiolate
	outer leaves: wax presence	weak	intermediate
	leaf: number of lobes	medium (Coaster, Topper)	medium (Coaster, Topper)
	outer leaves: foliage color (with wax, if present)	grey green	grey green
	outer leaves: foliage color (with wax, if present; RHS color chart value)	189A	189A
	leaf blade: color	grey green (Bishop)	grey green (Bishop)
	leaf blade: intensity of color	dark	dark
	leaf blade: anthocyanin coloration	absent (Claudia, Embassy)	absent (Claudia, Embassy)

(continued)

	Characteristic	RX05991199CMS	Ironman
	leaf blade: undulation of margin	weak (Beaufort, Early Pack, Laser, Paladin)	weak (Beaufort, Early Pack, Laser, Paladin)
	leaf blade: dentation of margin	weak (Galaxy)	weak (Galaxy)
	outer leaves: leaf shape	elliptic	elliptic
	outer leaves: leaf base	blunt	blunt
	outer leaves: leaf apex	blunt	blunt
	outer leaves: leaf margins	slightly wavy	slightly wavy
	outer leaves: leaf veins	intermediate	intermediate
	outer leaves: midrib	slightly raised	not raised
	leaf blade: blistering	medium (Medium Late 145, Skiff)	medium (Medium Late 145, Skiff)
	outer leaves: attitude (leaf angle from ground)	semi-erect (35-55 degrees)	semi-erect (35-55 degrees)
	Characteristic	**RX05991199CMS**	**Ironman**
	leaf: attitude (at beginning of head formation)	semi-erect (Arcadia, Asti, Civet, Claudia)	semi-erect
	outer leaves: torsion of leaf tip	none	none
	outer leaves: profile of upper side of leaf	planar	planar
F	Head		
	length of branching at base (excluding stem)	very short (Viola)	very short (Viola)
	diameter at widest point (at market maturity)	15.2 cm	14.7 cm
	depth (at market maturity)	9.7 cm	9.4 cm
	weight, market trimmed (at market maturity)	439.9 gm	400.3 gm
	color	grey green (Brigadeer, Galaxy)	grey green (Brigadeer, Galaxy)
	intensity of color	medium	medium
	RHS color chart value for head color	189B	189B
	anthocyanin coloration	absent (Early White Sprouting)	present (Brigadeer, Shogun, Viola)
	shape (at market maturity)	transverse elliptic (Buccaneer, Futura)	transverse elliptic (Buccaneer, Futura)
	dome shape (at market maturity)	domed	domed
	size (at market maturity) for US Exhibit C only **choice	medium** (Dundee, Early Man)	medium** (Dundee, Early Man)
	compactness / firmness (at market maturity)	medium (Late Corona)	short pedicels / tight / firm (Captain)

(continued)

F	Head		
	surface knobbling (at market maturity)	fine (Apollo, Brigadeer)	medium (Southern Comet)
	texture	medium (Clipper, Coaster)	fine (Auriga, Bishop, Green Top)
	bead size (at market maturity)	medium	small
	flower buds (at market maturity)	even in size	even in size
	Characteristic	**RX05991199CMS**	**Ironman**
	anthocyanin coloration of leaf axils (at market maturity)	absent	absent
	anthocyanin coloration of leaf veins (at market maturity)	absent	absent
	anthocyanin coloration of leaf blade (at market maturity)	absent	absent
	anthocyanin coloration of entire plant (at market maturity)	absent	absent
	anthocyanin coloration of leaf petiole (at market maturity)	absent (Claudia, Embassy)	absent (Claudia, Embassy)
	color of head leaves (at market maturity)	green	green
	RHS color chart value for the color of head leaves	N189B	N189B
	bracts	absent (Gem, Orion)	absent (Gem, Orion)
	secondary heads (at market maturity)	combination, present** (Marathon, Tribute, Late Purple Sprouting)	present, combination
	prominence of secondary heads (at market maturity)	intermediate (Citation)	weak
	number of secondary heads (at market maturity)	4	2
G	Flower		
	Color **choice for UPOV TG only	yellow** (Brigadeer, Orion)	yellow** (Brigadeer, Orion)
	intensity of yellow color	medium (Capitol, Corvet)	medium (Capitol, Corvet)
	color	4B	4B
	stalk color	green	green
	RHS color chart value for flower stalk color	138B	138B
	male sterility	present (Chevalier, Montop)	present (Chevalier, Montop)
*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention.			

Table 3: Physiological and Morphological Characteristics of Line BRM 53-3934 SI

	Characteristic	BMR53-3934SI	Sibsey
A	Region of Adaptation	NW Europe	NW Europe
B	Maturity, Spring Planted		
	days from direct seeding to 50% harvest	no direct seeding	no direct seeding
	days from transplanting to 50% harvest	76	54
	transplant date	10-May-10	10-May-10
	length of harvest period in days	16	5
	first harvest date	17-Jul-10	1-Jul-10
	last harvest date	1-Aug-10	5-Jul-10
	harvest season (main crop at 50% harvest)	summer	summer
	time of harvest maturity (50% of plants)	very late (Late Purple Sprouting)	very early (Earlyman, Primor)
	time of beginning of flowering (50% of plants with at least 10% flowers) **choice for UPOV TG only	late** (Shogun, Viola)	early** (Clipper, Southern Comet)
C	Seedling		
	cotyledon color	medium green	medium green
	RHS color chart value for seedling cotyledon color	147B	147B
	cotyledon anthocyanin	weak	intermediate
	hypocotyl anthocyanin	weak	intermediate
D	Plant		
	plant height in centimeters from soil line to top of leaves	51.7 cm	55.0 cm
	head height in centimeters from soil line to top of leaves	30.3 cm	50.0 cm
	height at harvest maturity	medium (Coaster)	medium (Coaster)
	number of stems	one (Ramoso Calabrese, Shogun)	one (Ramoso Calabrese, Shogun)
	branches	medium	many
	habit	compact	compact
	market class	fresh market	fresh market
	life cycle	annual	annual
	type of variety	inbred	first generation hybrid
E	Leaves		
	outer leaves: number of leaves per plant (at harvest)	20	12
	outer leaves: width (at midpoint of plant including petiole)	21.8 cm	18.9 cm

(continued)

E	Leaves		
	leaf: width	medium (Buccaneer, Green Belt)	narrow (Arcadia, Brigadeer)
	outer leaves: length (at midpoint of plant including petiole)	45.3 cm	39.1 cm
	leaf: length (including petiole)	medium (Brigadeer, Sumosun)	short (Dandy Early, Emperor)
	outer leaves: petiole length	20.2 cm	16.0 cm
	petiole: length	medium (Emperor, Ramoso Calabrese)	short (High Sierra, Padovano)
	outer leaves: leaf ratio - length/width	2:1	2:1
	outer leaves: leaf attachment	petiolate	petiolate
	outer leaves: wax presence	intermediate	intermediate
	leaf: number of lobes	medium (Coaster, Topper)	medium (Coaster, Topper)
	outer leaves: foliage color (with wax, if present)	blue green	grey green
	outer leaves: foliage color (with wax, if present; RHS color chart value)	N198A	189A
	Characteristic	**BMR53-3934SI**	**Sibsey**
	leaf blade: color	blue green (Citation, Esquire, Symphony)	grey green (Bishop)
	leaf blade: intensity of color	medium	medium
	leaf blade: anthocyanin coloration	absent (Claudia, Embassy)	absent (Claudia, Embassy)
	leaf blade: undulation of margin	weak (Beaufort, Early Pack, Laser, Paladin)	strong (Aikido, Marathon, Samurai)
	leaf blade: dentation of margin	weak (Galaxy)	weak (Galaxy)
	outer leaves: leaf shape	elliptic	elliptic
	outer leaves: leaf base	blunt	blunt
	outer leaves: leaf apex	blunt	blunt
	outer leaves: leaf margins	slightly wavy	very wavy
	outer leaves: leaf veins	intermediate	thin
	outer leaves: midrib	slightly raised	not raised
	leaf blade: blistering	absent or very weak (Buccaneer, Colibri)	absent or very weak (Buccaneer, Colibri)
	outer leaves: attitude (leaf angle from ground)	semi-erect (35-55 degrees)	semi-erect (35-55 degrees)
	leaf: attitude (at beginning of head formation)	semi-erect (Arcadia, Asti, Civet, Claudia)	semi-erect
	outer leaves: torsion of leaf tip	none	weak

(continued)

	Characteristic	BMR53-3934SI	Sibsey
	outer leaves: profile of upper side of leaf	concave	convex
F	Head		
	length of branching at base (excluding stem)	short (Brigadeer, Buccaneer Emperor)	medium (Capitol, Green Duke, Perseus)
	diameter at widest point (at market maturity)	11.4 cm	13.7 cm
	depth (at market maturity)	10.4 cm	10.9 cm
	weight, market trimmed (at market maturity)	164.3 gm	182.7 gm
	color	light green; grey-green (Brigadeer, Galaxy)	grey-green (Brigadeer, Galaxy)
	Characteristic	**BMR53-3934SI**	**Sibsey**
	intensity of color	medium	medium
	RHS color chart value for head color	189A/144A	N189B
	anthocyanin coloration	absent (Early White Sprouting)	absent (Early White Sprouting)
	shape (at market maturity)	circular (Esquire)	transverse elliptic (Buccaneer, Futura)
	dome shape (at market maturity)	domed	domed
	size (at market maturity) for US Exhibit C only **choice	very small (Early Purple Sprouting)	small** (Orbit, Scorpio)
	compactness / firmness (at market maturity)	long pedicels / loose (Caravel)	medium (Late Corona)
	surface knobbling (at market maturity)	medium (Southern Comet)	fine (Apollo, Brigadeer)
	texture	fine (Auriga, Bishop, Green Top); coarse (Citation)	fine (Auriga, Bishop, Green Top)
	bead size (at market maturity)	small / medium / large	small
	flower buds (at market maturity)	uneven in size (cateye)	even in size
	anthocyanin coloration of leaf axils (at market maturity)	absent	absent
	anthocyanin coloration of leaf veins (at market maturity)	absent	absent
	anthocyanin coloration of leaf blade (at market maturity)	absent	absent
	anthocyanin coloration of entire plant (at market maturity)	absent	absent
	anthocyanin coloration of leaf petiole (at market maturity)	absent (Claudia, Embassy)	absent (Claudia, Embassy)
	color of head leaves (at market maturity)	green	green

(continued)

	Characteristic	BMR53-3934SI	Sibsey
	RHS color chart value for the color of head leaves	N189B	189A
	bracts	present (Ramoso Calabrese)	absent (Gem, Orion)
	secondary heads (at market maturity)	basal, present** (Marathon, Tribute, Late Purple Sprouting)	combination
	prominence of secondary heads (at market maturity)	intermediate (Citation)	intermediate (Citation)
	number of secondary heads (at market maturity)	3	4
G	Flower		
	Color **choice for UPOV TG only	yellow** (Brigadeer, Orion)	yellow** (Brigadeer, Orion)
	intensity of yellow color	dark (Gem, Orion)	dark (Gem, Orion)
	color	4A	4A
	stalk color	green	green
	RHS color chart value for flower stalk color	138B	138B
	male sterility	absent (Marathon)	present (Chevalier, Montop)
*These are typical values. Values may vary due to environment. Other values that are substantially equivalent are also within the scope of the invention.			

[0058] The MSB content of hybrid RX 05991199 relative to the hybrid "Heritage" was the subject of an objective analysis. The results of the analysis are presented below.

Table 4: Analysis of MSB Content of RX 05991199

Varieties	Least-Squares Means for MSB (micromoles/gm/FW)
RX 05991199	3.5833927
Heritage	1.6311887

[0059] A head-to-head analysis was also made of glucoraphanin (MSB) content of RX 05991199 relative to the hybrid Ironman in multiple locations and plantings. The results are presented below.

Table 5: Analysis of Glucoraphanin Content of RX 05991199 Relative to Hybrid

				calculated in μmol per gDW	
Variety	**Country**	**SubRegion**	**Planting**	**Glucoiberin**	**Glucoraphanin**
Ironman	Italy	South	A	2.1	3.9
RX 05991199	Italy	South	A	5.0	20.0
RX 05991199	Italy	South	A	1.5	6.7
Ironman	Spain	South	B	2.1	3.1
RX 05991199	Spain	South	B	3.5	13.4

(continued)

Variety	Country	SubRegion	Planting	Glucoiberin	Glucoraphanin
Ironman	Italy	South	C	3.2	9.2
Ironman	Italy	South	C	3.6	9.1
Ironman	Italy	South	C	3.4	10.3
Ironman	Italy	South	C	3.3	10.8
RX 05991199	Italy	South	C	4.7	20.7
RX 05991199	Italy	South	C	5.4	22.3
RX 05991199	Italy	South	C	5.5	26.0
RX 05991199	Italy	South	C	4.7	22.9
Ironman	Spain	South	D	1.9	5.5
Ironman	Spain	South	D	2.8	9.6
Ironman	Spain	South	D	2.4	5.9
RX 05991199	Spain	South	D	5.1	21.7
RX 05991199	Spain	South	D	5.5	24.8
RX 05991199	Spain	South	D	5.1	25.7
Ironman	UK	North	A	2.5	9.8
Ironman	UK	North	A	3.1	12.8
RX 05991199	UK	North	A	3.8	21.3
RX 05991199	UK	North	A	3.4	24.4
Ironman	UK	North	B	0.9	7.1
Ironman	UK	North	B	1.9	12.1
RX 05991199	UK	North	B	2.4	11.9
RX 05991199	UK	North	B	3.0	16.0
Ironman	UK	North	C	1.8	5.8
Ironman	UK	North	C	1.9	5.8
RX 05991199	UK	North	C	3.1	14.1
RX 05991199	UK	North	C	3.3	15.1
Ironman	UK	North	D	1.1	6.2
Ironman	UK	North	D	0.9	6.2
RX 05991199	UK	North	D	3.2	17.2
RX 05991199	UK	North	D	3.1	16.6
ironman	Italy	South	F	0.9	5.1
ironman	Italy	South	F	1.0	5.3
RX 05991199	Italy	South	F	3.8	24.5
RX 05991199	Italy	South	F	3.6	25.1
ironman	Italy	South	A	0.9	4.3
ironman	Italy	South	A	1.0	5.1
RX 05991199	Italy	South	A	4.6	23.5
RX 05991199	Italy	South	A	4.0	22.4

(continued)

Variety	Country	SubRegion	Planting	Glucoiberin	Glucoraphanin
ironman	Spain	South	B	1.5	6.0
ironman	Spain	South	B	2.4	11.2
RX 05991199	Spain	South	B	4.0	20.7
RX 05991199	Spain	South	B	4.4	22.1
ironman	Spain	South	C	1.9	8.3
ironman	Spain	South	C	1.7	8.8
RX 05991199	Spain	South	C	4.1	24.3
RX 05991199	Spain	South	C	4.3	22.4
Ironman	Spain	South	D	1.2	5.5
Ironman	Spain	South	D	0.9	4.9
RX 05991199	Spain	South	D	3.5	19.0
RX 05991199	Spain	South	D	2.8	17.5
Ironman	Italy	South	E	0.9	7.6
Ironman	Italy	South	E	0.8	6.7
RX 05991199	Italy	South	E	3.0	24.4
RX 05991199	Italy	South	E	1.8	23.3
Ironman	Spain	South	F	0.8	5.0
Ironman	Spain	South	F	0.6	3.8
RX 05991199	Spain	South	F	2.9	17.1
RX 05991199	Spain	South	F	3.0	19.3
Ironman	Spain	South	G	1.0	6.3
Ironman	Spain	South	G	0.9	6.2
RX 05991199	Spain	South	G	3.4	20.8
RX 05991199	Spain	South	G	3.0	19.9
Ironman	Spain	South	H	2.5	14.7
Ironman	Spain	South	H	2.3	14.1
RX 05991199	Spain	South	H	3.6	21.9
RX 05991199	Spain	South	H	4.0	23.0
Ironman	UK	North	B	0.9	6.2
Ironman	UK	North	B	1.0	6.1
RX 05991199	UK	North	B	4.2	20.9
RX 05991199	UK	North	B	3.6	18.6
Ironman	UK	North	C	2.1	6.3
Ironman	UK	North	C	1.9	6.4
RX 05991199	UK	North	C	4.7	15.1
RX 05991199	UK	North	C	4.4	15.5
Ironman	UK	North	D	1.7	11.3
Ironman	UK	North	D	1.1	6.3

(continued)

Variety	Country	SubRegion	Planting	Glucoiberin	Glucoraphanin
RX 05991199	UK	North	D	4.1	22.0
RX 05991199	UK	North	D	2.7	14.6
Ironman	UK	North	E	2.1	11.9
RX 05991199	UK	North	E	5.3	24.2
Ironman	UK	North	F	1.2	8.0
Ironman	UK	North	F	1.5	8.7
RX 05991199	UK	North	F	4.2	19.9
RX 059911 99	UK	North	F	4.5	21.9
Ironman	UK	North	G	1.0	6.1
Ironman	UK	North	G	1.0	6.7
RX 05991199	UK	North	G	2.7	16.8
RX 05991199	UK	North	G	2.8	17.7
Ironman	UK	North	H	1.0	6.7
Ironman	UK	North	H	0.8	5.8
RX 05991199	UK	North	H	3.4	19.2
RX 05991199	UK	North	H	3.9	23.1
Ironman	Spain	South	A	0.6	4.0
Ironman	Spain	South	A	0.9	4.2
Ironman	Spain	South	A	0.8	3.7
RX 05991199	Spain	South	A	2.6	13.3
RX 05991199	Spain	South	A	2.7	14.1
RX 05991199	Spain	South	A	2.8	13.4
Ironman	Italy	South	B	1.4	7.3
Ironman	Italy	South	B	1.2	8.2
RX 05991199	Italy	South	B	4.9	26.6
RX 05991199	Italy	South	B	3.5	18.7
Ironman	Spain	South	C	1.1	5.9
Ironman	Spain	South	C	1.0	4.8
Ironman	Spain	South	C	1.0	5.5
RX 05991199	Spain	South	C	3.3	16.0
RX 05991199	Spain	South	C	2.7	13.0
RX05991199	Spain	South	C	2.9	11.7
Ironman	Italy	South	D	1.1	5.9
Ironman	Italy	South	D	1.1	5.3
RX 05991199	Italy	South	D	2.9	16.0
RX 05991199	Italy	South	D	3.9	21.4
Ironman	Spain	South	E	0.9	4.3
Ironman	Spain	South	E	1.0	3.9

(continued)

Variety	Country	SubRegion	Planting	Glucoiberin	Glucoraphanin
Ironman	Spain	South	E	1.1	4.8
RX 05991199	Spain	South	E	3.6	21.2
RX 05991199	Spain	South	E	2.2	16.5
RX 05991199	Spain	South	E	2.5	15.5
Ironman	Spain	South	F	1.1	4.6
Ironman	Spain	South	F	0.8	4.4
Ironman	Spain	South	F	1.0	4.5
RX 05991199	Spain	South	F	1.5	9.7
RX 05991199	Spain	South	F	2.3	15.4
RX 05991199	Spain	South	F	2.4	15.3
Ironman	Spain	South	G	1.2	4.4
Ironman	Spain	South	G	1.4	5.6
Ironman	Spain	South	G	1.0	3.4
RX 05991199	Spain	South	G	2.9	16.7
RX 05991199	Spain	South	G	2.8	18.7
RX 05991199	Spain	South	G	3.0	15.9
Ironman	UK	North	L	0.7	8.2
Ironman	UK	North	L	0.8	9.8
Ironman	UK	North	L	0.8	10.3
RX 05991199	UK	North	L	1.6	15.0
RX 05991199	UK	North	L	1.9	17.1
RX 05991199	UK	North	L	1.9	18.1
Ironman	UK	North	M	0.2	4.9
Ironman	UK	North	M	0.3	4.5
Ironman	UK	North	M	0.3	5.2
RX 05991199	UK	North	M	1.1	8.5
RX 05991199	UK	North	M	1.6	9.8
RX 05991199	UK	North	M	1.3	10.0

EXAMPLE 4

Use of Genetic Markers to Identify and Track Reduced Introgressions

[0060] Genetic marker assays were developed to genotype for Myb28 and ELONG alleles. The assays thus permit identification of a reduced introgression in accordance with the invention as well as marker assisted introduction of the reduced introgression into any other genotype.

A. Markers for detection of ELONG

[0061] A marker designated QTL1-BoGLS-ELONG was developed and permits detection of the presence or absence of a *B. villosa* ELONG allele. This marker can be detected using the primer pair AF399834F2: 5'-cggattttcaaattttctcg-3' (SEQ ID NO:1) and AF399834R2: 5'-atttcgcatgaccactaggc-3' (SEQ ID NO:2). To detect the marker, plates were loaded

with 20ng DNA template (sample) in a 2μL volume. 3μL master mix (0.437μL water, 2.5μL Q PCR (ROX) mix, 0.063uL assay mix) was added to each well for a final volume of 5μL. PCR conditions were as follows: 95°C for 15 min then 40 cycles of 95°C for 15 sec, 60°C for 1 min. FIG. 4 shows results of an assay for different ELONG alleles using the marker. The V allele is indicative of the *B. villosa* ELONG allele, while A, B and C alleles are examples of alleles found in broccoli without a *B. villosa* ELONG allele.

B. Marker for the detection of Myb28

[0062] The identification of genetic polymorphisms in Myb28 alleles and their use as genetic markers is described in U.S. Provisional Appln. Ser. No. 61/700,731, filed concurrently herewith, the disclosure of which is incorporated herein by reference in its entirety. Specifically, sequence alignments were described therein between *B. oleracea* alleles and the corresponding Myb28 sequences to identify polymorphisms that can be used for marker based selection for a Myb28 allele of choice. The results are shown in FIG. 5, which is an alignment between a consensus sequence of the *Myb28* locus from *B. villosa* contained in broccoli variety FT69, and a consensus sequence of the corresponding locus from broccoli without increased level of glucosinolate, *e.g. B. oleracea,* (Oleracea). Shown are 26 polymorphisms (*e.g.* single feature polymorphisms (SFPs) - of which there are 16 SNPs and 10 indels) detected in a sequence with a total length of 2202 bp. Any of these or other identified polymorphisms may be used as genetic markers for the presence or absence of a desired Myb28, including from B. *oleracea* or *B. villosa.*

[0063] A TaqMan assay (NBOLI009111370) was designed based on one of the sequence polymorphisms identified, as follows:

NBOLI009111370 sequence (SEQ ID NO:3):

GACCACCTAAAGACAAGAATAGTGAAAGAGATAAGATGGAAGACCAAAGTT
AATCAAATTTATTTTGAAGCTTTT[C/T]TATGGAATAGAGACTAAAATGATGT
GTGCTATTGCAATTTTTAGTCACATATTGCTAATCAAACACATATTTTGCATC
AGAGAATTGTCAAATACATGAAAAAAATAAAGAATAATTTTT

Forward primer (SEQ ID NO:4): GTGAAAGAGATAAGATGGAAGACCAAAGT
Reverse primer(SEQ ID NO:5): GTGACTAAAAATTGCAATAGCACACATCA
Vic probe (SEQ ID NO:6): CTATTCCATAGAAAAGC
Fam probe (SEQ ID NO:7): CTATTCCATAAAAAAGC

[0064] The assay was carried out using standard procedures as follows: Load plates with 20ng DNA template in 5μL volume. Add 10μL master mix (2 parts each of 1X PCR mix, 0.437μL water, 2.5μL Q PCR (ROX) mix, 0.063μL assay mix, 2μL primers at 5ng/μL) to each well for a final volume of 15μL.

[0065] PCR conditions are as follows: 50°C for 2 min followed by 95°C for 2 min then 40 cycles of 95°C for 15 sec, 60°C for 1 min.

Claims

1. A *Brassica oleracea* plant comprising a *Myb28* allele from *Brassica villosa* and lacking an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein the *Myb28* allele confers elevated glucosinolates to said plant when compared to a plant that lacks the *Myb28* allele, wherein said ELONG allele is detectable by SEQ ID NO:1 and SEQ ID NO:2, and wherein said *Myb28* allele comprises SEQ ID NO:6, and wherein a sample of seed comprising a chromosomal segment that comprises the *Myb28* allele from *Brassica villosa* and lacking an ELONG allele from *Brassica villosa* genetically linked to said *Myb28* allele, wherein the segment confers elevated glucosinolates relative to a plant lacking the *Myb28* allele, was deposited under ATCC Accession Number PTA-13165.

2. The plant of claim 1, wherein:

   (a) the plant is a broccoli plant;
   (b) the plant is inbred;
   (c) the plant is hybrid;

(d) the plant is homozygous for said *Myb28* allele from *Brassica villosa*; or
(e) the plant is heterozygous for said *Myb28* allele from *Brassica villosa.*

3. The plant of claim 1, wherein the ELONG allele is from *Brassica oleracea.*

4. A plant part of the plant of claim 1.

5. The plant part of claim 4, wherein the part is a leaf, a ovule, a floret, pollen, a head, or a cell.

6. A seed that produces the plant of claim 1.

Patentansprüche

1. *Brassica* oleracea-Pflanze, die ein Myb28-Allel aus *Brassica villosa* umfasst und der ein ELONG-Allel aus *Brassica villosa* fehlt, welches genetisch mit dem Myb28-Allel verknüpft ist, wobei das Myb28-Allel der Pflanze im Vergleich zu einer Pflanze, der das Myb28-Allel fehlt, eine Erhöhung der Glucosinolate verleiht, wobei das ELONG-Allel durch SEQ ID NO:1 und SEQ ID NO:2 nachweisbar ist und wobei das Myb28-Allel SEQ ID NO: 6 umfasst, und wobei eine Saatgutprobe unter der ATCC-Zugangsnummer PTA-13165 hinterlegt wurde, die ein chromosomales Segment aufweist, welches das Myb28-Allel aus *Brassica villosa* umfasst und der ein ELONG-Allel aus *Brassica villosa* fehlt, das genetisch mit dem Myb28-Allel verbunden ist, wobei das Segment Erhöhung der Glucosinolate im Vergleich zu einer Pflanze verleiht, der das Myb28-Allel fehlt.

2. Pflanze gemäß Anspruch 1, wobei:

(a) die Pflanze eine Brokkoli Pflanze ist;
(b) die Pflanze ingezüchtet ist;
(c) die Pflanze ein Hybrid ist;
(d) die Pflanze für das Myb28-Allel aus *Brassica villosa* homozygot ist; oder
(e) die Pflanze für das Myb28-Allel aus *Brassica villosa* heterozygot ist.

3. Pflanze gemäß Anspruch 1, wobei das ELONG-Allel aus *Brassica oleracea* stammt.

4. Teil der Pflanze gemäß Anspruch 1.

5. Teil der Pflanze gemäß Anspruch 4, wobei es sich bei dem Teil um ein Blatt, eine Eizelle, eine Blüte, einen Pollen, einen Kopf oder eine Zelle handelt.

6. Samen, der die Pflanze gemäß Anspruch 1 hervorbringt.

Revendications

1. Plante de *Brassica oleracea* comprenant un allèle *Myb28* provenant de *Brassica villosa* et dépourvue d'un allèle ELONG de *Brassica villosa* lié génétiquement audit allèle *Myb28,* l'allèle *Myb28* conférant des taux élevés de glucosinolates à ladite plante en comparaison d'une plante qui est dépourvue de l'allèle *Myb28,* dans laquelle ledit allèle ELONG est détectable par les séquences SEQ ID NO: 1 et SEQ ID NO: 2, et dans laquelle ledit allèle *Myb28* comprend la séquence SEQ ID NO: 6, et dans laquelle un échantillon de semence comprenant un segment chromosomique qui comprend l'allèle *Myb28* provenant de *Brassica villosa* et dépourvu d'un allèle ELONG de *Brassica villosa* lié génétiquement audit allèle *Myb28,* le segment conférant des taux élevés de glucosinolates par rapport à une plante dépourvue de l'allèle *Myb28,* a été déposé sous le numéro d'enregistrement ATCC PTA-13165.

2. Plante selon la revendication 1, dans laquelle :

(a) la plante est une plante de brocoli ;
(b) la plante est de lignée pure ;
(c) la plante est hybride ;
(d) la plante est homozygote pour ledit allèle *Myb28* provenant de *Brassica villosa* ; ou

(e) la plante est hétérozygote pour ledit allèle *Myb28* provenant de *Brassica villosa.*

3. Plante selon la revendication 1, dans laquelle l'allèle ELONG est de *Brassica oleracea.*

4. Partie de plante de la plante selon la revendication 1.

5. Partie de plante selon la revendication 4, dans laquelle la partie est une feuille, un ovule, un fleuron, du pollen, une inflorescence, ou une cellule.

6. Semence qui produit la plante selon la revendication 1.

FIG. 1



FIG. 2



FIG. 3



FIG. 4

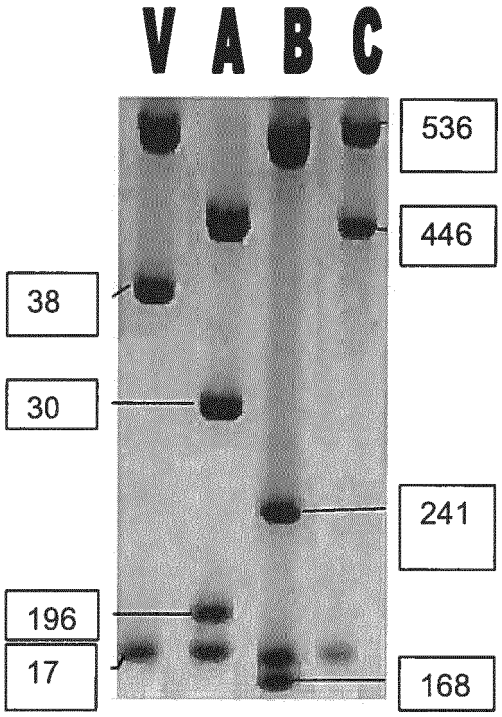


FIG. 5

The B.oleracea lines sequenced to create this consensus are:
GD33
breeder line 560216
breeder ID field number 2153.

The lines that contain the FT69 allele and were used to create this consensus are:
Breeder line 560526
Breeder line 580333
Breeder line BRM 51-1162
Breeder line BRM51-1210

```
FT69      1    GAAAATCACAGTTCACGCCTCTTACTCCATGAGCTTCTCTATTCTCATCC
Oleracea 1    GAAAATCACAGTTCACGCCTCTTACTCCATGAGCTTCTCTATTCTCATCC

FT69      51   TAGTGTTATAATCTTGCAAACACATATAGAAAGCAAGATTTGGAGTGTAC
Oleracea 51   TAGTGTTATAATCTTGCAAACACATATAGAAAGCAAGGTTTGGAGTGTAC

FT69      101  GAGAAAAACATGAAAACACCTAGAAGCTCTGTGGGTAAGACCCAAGAGCG
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FT69      151  TTTCTCGATTAGTTTCATATACAGATGCATCAGAGTTCTCATCAACCGAT
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FT69      201  CTACTTCTTTCTTATCTTATTAGAAAAAAAAAAATCCTATCAAAATTTACT
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FT69      251  TTCCTGCAAGTATATTTTTCTTTACATTTTCATTTTCTTGAGTGTTATTT
Oleracea 251  TTCCTGCAAGTATATTTTTCTTTACATTTTCATTTTCTTGAGTGTTATTT

FT69      301  GAGTGAAGTTATATTAAAATATT::::::::GTTCATATATATCGAAAAT
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FT69      351  GTCAAGAAAGCCATGTTGTGTCGGAGAAGGGCTGAAGAAAGGGGCATGGA
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FT69      401  CCACCGAGGAAGATAAGAAACTCATCTCTTACATCCATGAACATGGAGAA
Oleracea 401  CCACCGAGGAAGATAAGAAACTCATCTCTTACATCCATGAACATGGAGAA

FT69      451  GGAGGCTGGCGCGACATTCCTCAAAAAGCTGGTTAATATCTATTATATAT
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FT69      501  TTTTTGGTAAATTTTTAAAAC::ATATATGTTTGTTTGGTATTTGATGTA
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FT69      551  TGAAAGTTTTATATTGAATGTGGTGTTTTACTAGGATTGAAAAGGTGTGG
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FT69      601  AAAGAGTTGCAGACTGCGATGGACTAACTACCTAAAACCTGAGATCAAAA
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FT69      651  GAGGCGAGTTTAGTTCAGAGGAGGAACAGATTATCATCATGCTCCATGCT
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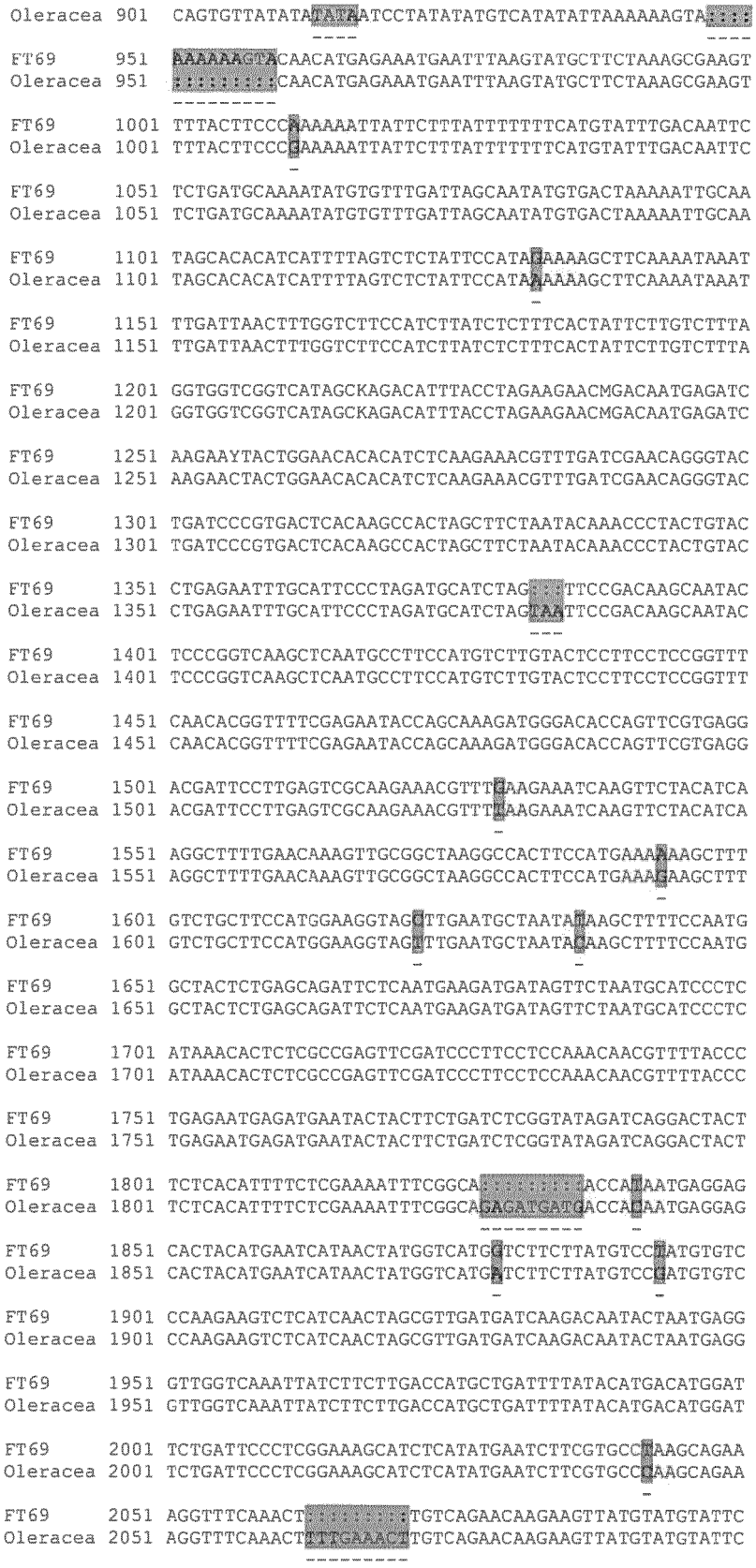
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Oleracea 701  GCTCGTGGCAACAAGTACGTTTATTTTAGACCAAAAAAAAAACAAGTACGT

FT69      751  TTATTTTTAACAAAAAGGACGATTATATATTTT::TGTGTGTATGGATCC
Oleracea 751  TTATTTTTAACAAAAAGGACGATTATATATTTTTATGTGTGTATGGATCC

FT69      801  TCCAGTGATCATCATTCTAGTTTTCTCTTTTTTTTTTATACCGCAAACA
Oleracea 801  TCCAGTGATCATCATTCTAGTTTTCTCTTTTTTTTT::ATACCGCAAACA

FT69      851  AATTTCATTAGTAAAAAAAATTTAAAATTCCAAAGTCAATATTCAAAAACA
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FT69      901  CAGTGTTATATA::::ATCCTATATATGTCATATATTAAAAAAGTATATT
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Oleracea 901 CAGTGTTATATATATAATCCTATATATGTCATATATTAAAAAAGTA
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FT69 1951 GTTGGTCAAATTATCTTCTTGACCATGCTGATTTTATACATGACATGGAT
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FT69 2001 TCTGATTCCCTCGGAAAGCATCTCATATGAATCTTCGTGCCTAAGCAGAA
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FT69      2151 AGTTTAGGGTTTGTATAATATACAATAAGGGACGTTATCTTATAAAACGA
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FT69      2201 GG
Oleracea 2201 GG
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REFERENCES CITED IN THE DESCRIPTION

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