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(54) Title: BRASSICA NAPUS PLANTS HAVING ENHANCED BLACKLEG RESISTANCE

(57) Abstract: The present invention relates to the field of plant breeding. Provided is a Brassica napus plant comprising blackleg resistance locus BL160 (BL160 locus), a seed produced by the plant according to the present invention, a seed from which a plant according to present invention can be grown, a part of a plant according to the present invention, a method of identifying and/or selecting a plant or plant part according to the present invention, a method for producing a Brassica napus plant having a blackleg resistance phenotype and a method for enhancing the blackleg resistance phenotype of a Brassica napus plant. Further provided is the use of the BL160 locus for enhancing the blackleg resistance phenotype in a Brassica napus plant, and genetic markers specific for the BL160 locus and the use thereof for selecting a Brassica napus plant having an enhanced blackleg resistance phenotype.

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BRASSICA NAPUS PLANTS HAVING ENHANCED BLACKLEG RESISTANCE

The present invention relates to the field of plant breeding. Provided is a *Brassica napus* plant comprising blackleg resistance locus BL160 (BL160 locus). Further provided is a seed produced by the plant according to the present invention, a seed from which a plant according to present invention can be grown and a part of a plant according to the present invention. Further provided is a method of identifying and/or selecting a plant or plant part according to the present invention, a method for producing a *Brassica napus* plant having a blackleg resistance phenotype and a method for enhancing the blackleg resistance phenotype of a *Brassica napus* plant. Further provided is the use of the BL160 locus for enhancing the blackleg resistance phenotype in a *Brassica napus* plant. Further provided are genetic markers specific for the BL160 locus and the use thereof for selecting a *Brassica napus* plant having an enhanced blackleg resistance phenotype.

Background of the invention

Blackleg or stem canker is a major disease of *Brassica napus* L. (oilseed rape or Canola), causing annually major economic losses worldwide, in particular in Europe, Australia and North America. Blackleg is caused by the fungal pathogen *Leptosphaeria maculans* (anamorph *Phoma lingam* Tode ex. Fr.). *L. maculans* symptoms can develop on cotyledons, leaves, pods and stems. Leaf lesions develop after infection by wind dispersed ascospores and/or water (splash) dispersed conidiospores. Stem symptoms (or cankers) can arise through direct infection of the stems or through systemic growth of the fungus from leaf lesions, through the vascular tissue into the stem (Hammond et al. (1985), *Plant Pathology* 34: 557-565). Stem cankers may girdle the stem, which can lead to the lodging of plants and plant death. Less severe cankers can cause a restriction in water and nutrient flow, which in turn may lead to shrivelling of seeds and pods. Pod infection can lead to premature podshatter and seed infection.

The incorporation of blackleg resistance into *B. napus* cultivars is one of the major objectives in breeding programs worldwide. Although both the spraying of fungicides and cultural practices are used to reduce yield losses caused by blackleg infection, the most reliable method of control to date is genetic resistance. *Brassica napus* (2n=38, genome AACC) is an amphidiploid species, which originated from a spontaneous hybridization of *Brassica rapa* L. (syn. *B. campestris;* 2n=20, AA) and *Brassica oleracea* L. (2n=18, CC). *B. napus* contains the complete chromosome sets of these two diploid genomes.

Plant resistance is a powerful tool to combat blackleg disease. Blackleg resistance is assessed either in glasshouse or in field experiments. Further, it can be assessed at different stages of the plant development. When referring to blackleg resistance, normally different types of resistance are therefore distinguished depending on the plant stage and tissue assessed, such as seedling resistance ("early" resistance) and adult plant resistance ('late' or 'stem' resistance). Plant tissues analysed for resistance are for example cotyledons, leaves and stem bases. Genetical resistance to blackleg has been reported to be either monogenic (under control of a major gene) or polygenic (under control of several minor genes).

Resistance against the hemibiotrophic fungal pathogen *Leptosphaeria maculans* is governed largely by both race-non-specific and race-specific R resistance genes. A number of resistance loci have been mapped in *B. napus*. More than 15 R genes, identified in several Brassica species, have been reported to convey race-specific resistance against *L. maculans*. These R genes have been positioned in the A genome of *B. napus* or *B. rapa* (for example *Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm9, LepR1, LepR2, LepR3, LepR4*) or the B genome of *B. juncea*, *B. carinata* and *B. nigra* (*Rlm6*, *Rlm10*, *LMJR1, LMJR2,* and *rjlm2*) via linkage mapping (Larkan et al. (2016) Single R Gene Introgression Lines for Accurate Dissection of the Brassica - Leptosphaeria Pathosystem. Front. Plant Sci. 7:1771. doi: 10.3389/fpls.2016.01771, Cantila et al. (2021) Recent Findings Unravel Genes and Genetic Factors Underlying Leptosphaeria maculans Resistance in Brassica napus and Its Relatives. Int J Mol Sci. 2021 Jan; 22(1): 313. doi: 10.3390/ijms22010313). One R gene was also identified on the C genome, called *Rlm13* (Raman et al. (2021) The *Rlm13* Gene, a New Player of *Brassica napus–Leptosphaeria maculans* Interaction Maps on Chromosome C03 in Canola. Front. Plant Sci. 12:654604. doi: 10.3389/fpls.2021.654604).

The major blackleg resistance gene *Rlm6* was introduced from *Brassica juncea* into *Brassica napus* (Chèvre et al. (1997) Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 1: Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Genet. 95, 1104-111. doi: 10.1007/s001220050669).

Another publication is reporting the introgression of the resistance gene *Rlm6* from *B. juncea* 'Forge' into *B. napus* 'Topas DH16516' (Rashid et al. (2018) Development of molecular markers linked to the Leptosphaeria maculans resistance gene Rlm6 and inheritance of SCAR and CAPS markers in Brassica napus × Brassica juncea interspecific hybrids. Plant Breeding, 137(3), 402-411. doi: https://doi.org/10.1111/pbr.12587.

US 7,893,325 describes a *Brassica napus* plant, comprising on chromosome 8 a *Leptosphaeria maculans* resistance gene derived from *Brassica rapa*, wherein said resistance gene is associated with AFLP markers E32/M50-M362 and P34/M48-M283 on chromosome 8.

WO 2008/101343 A1 describes a method of conferring blackleg resistance to a plant comprising: introducing a nucleic acid molecule comprising a nucleic acid molecule encoding LepR3.

WO 2015/038469 A1 describes molecular markers for blackleg resistance gene *Rlm2* in *Brassica napus*.

WO 2015/038470 A1 describes molecular markers for blackleg resistance gene *Rlm4* in *Brassica napus*.

WO 2020/036950 A1 describes molecular markers for blackleg resistance gene *Rlm1* in *Brassica napus*.

WO2020/036954 A1 describes molecular markers for blackleg resistance gene *Rlm7* in *Brassica napus*.

The PCT patent application having application number PCT/US22/74073 describes molecular markers for blackleg resistance gene *Rlm3* in *Brassica napus*.

It is desirable to identify new genetic sources of resistance, methods for transferring these into varieties with high agronomic performance and methods for enhancing durability of resistance.

The present invention, including the different embodiments provided in the specification and claims, provides plants comprising blackleg resistance locus BL160 (BL160 locus), and methods and means for transferring the BL160 locus into plants as well as methods of detecting the presence/absence of the BL160 locus in plants.

Summary of the invention

The present invention provides a *Brassica napus* plant having enhanced resistance to blackleg, wherein said resistance is provided by blackleg resistance locus BL160 (BL160 locus), wherein said BL160 locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 6.

In addition, the present invention provides a seed produced by the *Brassica napus* plant according to the present invention wherein the seed comprises the BL160 locus as described herein. The present invention further provides a seed from which the *Brassica napus* plant according to the present invention can be grown. The present invention further provides a plant cell, tissue or plant part of the *Brassica napus* plant according to the present invention or of the seed according to the present invention, comprising the BL160 locus as described herein. The present invention further provides a haploid plant or dihaploid plant derived from the *Brassica napus* plant as provided by the present invention.

In addition, the present invention provides a method for identifying and/or selecting a *Brassica napus* plant or plant part comprising determining whether said plant or plant part comprises in its genome the BL160 locus as described herein.

In addition, the present invention provides a method for producing a *Brassica napus* plant having a blackleg resistance phenotype, said method comprising the step(s) of: (i) crossing a first *Brassica napus* plant and a second plant, wherein the first *Brassica napus* plant

comprises in its genome the BL160 locus as described herein; (ii) optionally harvesting seed from the crossing of (i) and selecting seed comprising the BL160 locus in its genome.

In addition, the present invention provides a method for enhancing the blackleg resistance phenotype of a *Brassica napus* plant, said method comprising introgressing the BL160 locus as described herein into said *Brassica napus* plant.

In addition, the present invention provides the use of the BL160 locus as described herein for enhancing the blackleg resistance phenotype in a *Brassica napus* plant.
In addition, the present invention provides the use of a genetic marker specific of the BL160 locus as described herein for selecting a *Brassica napus* plant having an enhanced blackleg resistance phenotype.

In addition, the present invention provides a method for the protection of a group of cultivated plants according to the present invention comprising a technically induced mutation which confers herbicide tolerance in a field wherein weeds are controlled by the application of a composition comprising one or more herbicidal active ingredients.

In addition, the present invention provides a method for stacking the BL160 locus and a further gene of interest, said method comprising introducing said other gene of interest in the genome of the *Brassica napus* plant according to the present invention at a genomic position which is genetically linked to the BL160 locus.

Detailed description of the present invention

The current invention is based on the identification of the blackleg resistance locus BL160 (BL160 locus) in Brassica.

The present invention provides a *Brassica napus* plant having enhanced resistance to blackleg, wherein said resistance is provided by blackleg resistance locus BL160 (BL160 locus), wherein said BL160 locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 6. Particularly, the present invention provides a *Brassica napus* plant having enhanced resistance to blackleg, wherein said resistance is provided by blackleg resistance locus BL160 (BL160 locus), wherein said BL160 locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6 identity to SEQ ID NO: 6.

The inventors found that *Brassica napus* plants comprising blackleg resistance locus BL160 (BL160 locus) comprised in an introgression fragment from *Brassica juncea* chromosome

B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 6 show a resistance response to the *L. maculans* pathogen, particularly to the *L. maculans* isolates 18SK100 ,15SK40, 12CC-111 and 21AB07. BL160 confers a different resistance profile compared to all known phenotyped Rlm genes including Rlm1, Rlm2, Rlm3, Rlm4, Rlm6, Rlm7 and Rlm9. Particularly, BL160 and Rlm6 show a resistance reaction against both 18SK100 and 15SK40. Isolate 19SK21 differentiates BL160 from Rlm6 by showing a susceptible and resistant reactions, respectively, to BL160 and Rlm6, whereas isolates 12CC-111 and 21AB07 differentiates BL160 from Rlm6 by showing a resistant and susceptible reactions, respectively.

"Blackleg" as used herein refers to the disease caused by the fungal pathogen *Leptosphaeria maculans* or *Phoma lingam* (anamorph). The definition encompasses both Tox^0 and Tox^+ isolates, irrespective of whether these may be found to belong to different species in later taxonomic studies.

Previously, *L. maculans* isolates were classified into different pathogenicity groups (PG), depending on their specific interactions with *B. napus* cultivars Westar, Glacier and Quinta (Mengistu et al. (1991), *Plant Disease* 75: 1279-1282). PG4 isolates cause sporulating lesions on all three cultivars, while PG3 isolates cause a resistance reaction on cotyledons of Quinta, and PG2 isolates cause a resistance reaction on cotyledons of Quinta and Glacier. PG1 isolates are nonpathogenic on these hosts. PG2, PG3 and PG4 isolates are also referred to as 'highly aggressive' or 'highly virulent' or 'strongly pathogenic' isolates, while PG1 isolates are referred to as 'non-aggressive' or 'non-virulent' or 'weakly pathogenic' in the literature, sometimes the highly aggressive group is also termed "A" while the weakly aggressive group is termed "NA" (Badawy and Hoppe (1989), *J Phytopathology* 127: 146-157). The highly aggressive group is distinguished from the weakly aggressive group by its production of toxins (Tox^+ isolates vs Tox^0 isolates). Tox^0 isolates have been found to cause necrosis of the pith, unaccompanied by external symptoms, and it has been suggested that the effect on yield loss caused by Tox^0 isolates has been underestimated (Johnson and Lewis (1994), *Plant Pathology* 43: 269-277). Tox^0 isolates are further distinguished into three groups, NA1, NA2 and NA3 and it has been suggested that NA1 isolates are predominant in Europe and NA2 isolates are more important in Canada (Gall et al. (1995), *Mycol Res* 99: 221-229). In recent years, researchers have moved away from the PG nomenclature system towards classifying *L. maculans* isolates using several isogenic lines containing individual R genes introgressed in the same background. This provides a better understanding of the diversity and classification of isolates based on a virulence and virulence genes in different isolates within *L maculans* populations (Larkan et al. 2016. Frontiers in Science.).

L maculans isolates used in the context of the present invention were collected from infested canola stubble from the field in Saskatchewan, Canada in 2015, 2018 and 2019. Small plant tissues were excised from plant tissues, surface sterilized in 5% bleach and cultured on V-8 Bacto Agar. Pure single spore isolates were obtained by sub-culturing diluted spores on potato dextrose agar (PDA). Individual spores were then excised along with a small piece of

PDA and sub-cultured back on V-8 Bacto Agar. Single spore cultures where then allocated the names to designate the collection year, province, and a sequential number at the end. Particularly, the following *Leptosphaeria maculans* isolates have been used in the context of the present invention: *Leptosphaeria maculans* isolate designated as 15SK40, a representative sample of which has been deposited under Accession Number IDAC 010323-01; *Leptosphaeria maculans* isolate designated as 18SK100, a representative sample of which has been deposited under Accession Number IDAC 010323-02; *Leptosphaeria maculans* isolate designated as 19SK07, a representative sample of which has been deposited under Accession Number IDAC 010323-03; *Leptosphaeria maculans* isolate designated as 19SK21, and a representative sample of which has been deposited under Accession Number IDAC 010323-04.

In one aspect, the resistant to *Leptosphaeria maculans* isolate designated 18SK100, a representative sample of which has been deposited under accession IDAC 010323-02 and/or resistant to *Leptosphaeria maculans* isolate designated 15SK40, a representative sample of which has been deposited under accession number IDAC 010323-01. In another aspect, the *Brassica napus* plant according to the present invention is also resistant to *Leptosphaeria maculans* isolate designated 12CC-111, and/or resistant to *Leptosphaeria maculans* isolate designated 21AB07. It was found in the context of the present invention that *Brassica napus* plants comprising the BL160 locus according to the present invention show a resistance response to the *L. maculans* pathogen, particularly to the *L. maculans* isolates 18SK100, 15SK40, 12CC-111 and 21AB07.

As used herein, "resistance response" or "enhanced resistance" or "improved resistance" of plants comprising a certain resistance locus or a resistance gene refers to a reduction in damage caused by fungal infection (such as with *Leptosphaeria maculans*) compared to damage caused on control plants. Damage can be assessed as, for example, the number and size of leaf symptoms, frequency and severity of stem symptoms, lodging of plants due to stem infection, etc. In particular, the reduction in damage is manifested in a reduced yield loss when plants are grown under disease pressure in the field, compared to control plants. Such reduction in yield loss can, for example, be due to the fact that the infection, reproduction, spread or survival of the fungus is reduced or prevented in plants with enhanced resistance. Enhanced resistance may also refer to plants that are completely resistant, i.e., plants on which no disease symptoms are found or plants which get the highest resistance scores in available blackleg scoring or rating assays, e.g., Khangura et al. (2003, Department of Agriculture, Western Australia, Farmnote No. 6/2003, ISSN 0726-934×).

For instance, a suitable (negative) control plant may be an isogenic plant derived from *Brassica napus* plant BL160 (NCIMB 44185) not comprising the BL160 locus as described herein. In general, it is understood that comparisons between different plants or plant lines or varieties involves growing a number of plants of a line (or variety) (e.g. at least 5 plants, preferably at least 10 plants per line) under the same conditions as the plants of one or more control plant lines (preferably wild type plants) and the determination of differences, preferably statistically significant differences, between the plant lines when grown under the

same environmental conditions. Preferably the plants are of the same line or variety. More preferably, the control plants are isogenic plants. The term "isogenic plant" refers to two plants which are genetically identical except for the resistance locus of interest or resistance gene of interest. For example, the suitable (negative) control plant may be *Brassica napus* cultivar (cv) Westar or *Brassica napus* cultivar Topas. Both cultivars are susceptible to blackleg (see Examples).

Enhanced resistance can also be assessed in bioassays carried out in controlled environments, such as growth chambers, but ideally are confirmed in field trials, as controlled environment assessments often do not reflect field conditions. This may be due to the fact that few, single spore isolates of the fungus are normally tested in bioassays, while in the field much larger variation in the pathogen population exists.

It is understood that environmental conditions, such as location, weather conditions and disease pressure, as well as individual perception of the person assessing disease symptoms, can have an effect on the scoring of blackleg resistance. Hence, variation in these factors in comparative tests should be minimized. Any other resistance ratings known in the art can be applied in accordance with this invention to compare the plants of the invention with control plants.

How to assess, whether a plant is resistant (or susceptible) is well known in the art. Further, it is described in the Examples section, see Example 2. For example, a scoring system can be applied using a scale from 1 to 9, with 1 being susceptible and 9 being resistant. Further, a scoring system is disclosed in Khangura et al. (2003, Department of Agriculture, Western Australia, Farmnote No. 6/2003, ISSN 0726-934×). Plants are inoculated with *L. maculans* 6-7 days after seeding and rated 14 days post inoculation using a 0-9 scale wherein plants obtaining a score of 0-5 are considered “Resistant” and plants obtaining a score of 6-9 are considered “Susceptible”; see also Example 2, below.

Accordingly, the BL160 locus as provided by the present invention and which is capable of conferring blackleg resistance is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6. The term “blackleg resistance locus BL160” or “BL160 locus”, as used herein, accordingly refers to the hereditary unit which confers enhanced resistance to blackleg, particularly enhanced resistance to *Leptosphaeria maculans*, more particularly enhanced resistance to *Leptosphaeria maculans* isolate designated 18SK100, a representative sample of which has been deposited under accession number IDAC 010323-02 and/or *Leptosphaeria maculans* isolate designated 15SK40, a representative sample of which has been deposited under accession number IDAC 010323-01. Additionally, the “blackleg resistance locus BL160” or “BL160 locus” confers enhanced resistance to *Leptosphaeria maculans* isolate designated as 12CC-111, and/or *Leptosphaeria maculans* isolate designated as 21AB07 when compared to a plant lacking the BL160 locus.

This resistance locus can be transferred to different varieties of *B. napus*, and even to different species of Brassica plants, e.g. *B. juncea*, e.g., using the molecular markers of this invention. In addition to the specific blackleg resistance phenotype, accordingly, the BL160 locus of the present invention is further defined in that it is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6.

The term "locus" (plural loci) means a specific place or places or a site on a chromosome where for example a gene or genetic marker is found. A “blackleg resistance locus” accordingly refers to the position on the chromosome where a “blackleg resistance gene” is located. This position can be identified by the location on the genetic map of a chromosome. Included in this definition is the fragment (or segment) of genomic DNA of the chromosome on which the blackleg resistance locus is located.

As is used herein, a QTL (quantitative trait locus) is a hereditary unit (often indicated by one or more molecular genomic markers) that occupies a specific location on a chromosome and that contains the genetic instruction for a particular phenotypic characteristics or trait in a plant. In contrast to a gene, the exact boundaries of a QTL are not known, but can be found without undue burden by a person skilled in the art by using fine mapping techniques well known in the art of genetic mapping and subsequent DNA sequencing routines. The QTL encodes at least one gene of which the expression, alone or in combination with other genes, results in the phenotypic trait being expressed, or that encodes at least one regulatory region that controls the expression of at least one gene the expression of which, alone or in combination with other genes, results in the phenotypic trait being expressed. A QTL may be defined by indicating its genetic location in the genome of the donor of the introgression that contains the QTL using one or more molecular genomic markers. These one or more markers, in turn, indicate a specific locus. Preferably, the BL160 locus of the present invention is a quantitative trait locus (also named herein as “blackleg resistance QTL BL160” or “BL160 QTL”).

Distances between loci are usually measured by frequency of crossing-over between loci on the same chromosome. The further apart two loci are, the more likely that a crossover will occur between them. Conversely, if two loci are close together, a crossover is less likely to occur between them. As a rule, one centimorgan (cM) is equal to 1% recombination between loci (markers). When a locus, preferably a QTL, can be indicated by multiple markers the genetic distance between the end-point markers is indicative of the size of the locus, respectively the QTL. Markers that define the QTL may be markers that are linked to the QTL or markers that are in linkage disequilibrium with the QTL. As used herein, the term "linked to” or "genetically linked" when used in the context of markers and/or genomic regions means that the two linked loci (e.g. a marker and a QTL) are separated on a genetic map by 10 cM or less (i.e meiotic recombination between the two linked loci occurs with a frequency of equal to or less than 10%), more preferably by 9 cM or less, 8 cM or less, 7 cM or less, 6 cM

or less, 5 cM or less, 4 cM or less, 3 cM or less, 2 cM or less, 1 cM or less, 0.75 cM or less, 0.5 cM or less, or even 0.25 cM or less. As used herein, the term "linkage disequilibrium" describes a non-random segregation of genetic loci or traits (or both).

The term "genome" relates to the genetic material of an organism. It consists of DNA. The genome includes both the genes and the non-coding sequences of the DNA.

The term "gene" means a (genomic) DNA sequence comprising a region (transcribed region), which is transcribed into a messenger RNA molecule (mRNA) in a cell, and an operably linked regulatory region (also described herein as regulatory sequence, e.g. a promoter). A gene may thus comprise several operably linked sequences, such as a promoter, a 5' leader sequence comprising e.g. sequences involved in translation initiation, a (protein) coding region (cDNA or genomic DNA) and a 3' non-translated sequence comprising e.g. transcription termination sites. Different alleles of a gene are thus different alternative forms of the gene, which may be in the form of e.g. differences in one or more nucleotides of the genomic DNA sequence (e.g. in the promoter sequence, the exon sequences, intron sequences, etc.), mRNA and/or amino acid sequence of the encoded protein. A gene may be an endogenous gene (in the species of origin) or a chimeric gene (e.g. a transgene or cis-gene). The "promoter" of a gene sequence is defined as a region of DNA that initiates transcription of a particular gene. Promoters are located near the genes they transcribe, on the same strand and upstream on the DNA. Promoters can be about 100-1000 base pairs long. In one aspect the promoter is defined as the region of about 1000 base pairs or more e.g. about 1500 or 2000, upstream of the start codon (i.e. ATG) of the protein encoded by the gene.

“Expression of a gene" refers to a process wherein a DNA region, which is operably linked to appropriate regulatory regions, particularly a promoter, is transcribed into an RNA, which is biologically active, i.e. which is capable of being translated into a biologically active protein or peptide or which is active itself (e.g. in posttranscriptional gene silencing or RNAi). The coding sequence may be in sense-orientation and encodes a desired, biologically active protein or peptide.

The terms "protein" and "polypeptide" are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3 - dimensional structure or origin. A "fragment" or "portion" of a protein may thus still be referred to as a "protein". An "isolated protein" is used to refer to a protein which is no longer in its natural environment, for example in vitro or in a recombinant bacterial or plant host cell.

The terms “peptide sequence” and “amino acid sequence” refer to the primary amino acid sequence of a protein or polypeptide.

The term "allele(s)" means any of one or more alternative forms of a gene at a particular locus, all of which alleles relate to one trait or characteristic at a specific locus. In a diploid cell of an organism, alleles of a given gene are located at a specific location, or locus (loci plural) on a chromosome. One allele is present on each chromosome of the pair of

homologous chromosomes. A diploid plant species may comprise a large number of different alleles at a particular locus. These may be identical alleles of the gene (homozygous) or two different alleles (heterozygous).

An allelism test is a test known in the art that can be used to identify whether two genes conferring the same trait are located at the same locus.

The word "trait" in the context of this application refers to the phenotype of the plant. When a plant shows the traits of the invention, its genome comprises the allele causing the trait of the invention. The plant, thus, has the hereditary unit of the invention. It is understood that when referring to a plant comprising the trait of the plant of the invention, reference is made to a *Brassica napus* plant comprising the improved blackleg resistance trait of the present invention.

In one aspect, the BL160 locus as comprised in the *Brassica napus* plant according to the present invention is comprised in an introgression fragment, preferably in an introgression fragment from *Brassica juncea*. Said introgression fragment comprising the BL160 locus may be as present in, or as obtainable from, or as obtained from, or as comprised in the genome of a *Brassica napus* plant designated BL160, a representative sample of which has been deposited under accession number NCIMB 44185. In a further aspect, the introgression fragment comprising the BL160 locus may be a functional fragment of the introgression fragment as present in, or as obtainable from, or as obtained from, or as comprised in the genome of a *Brassica napus* plant designated BL160, a representative sample of which has been deposited under accession number NCIMB 44185. A “functional fragment” of the introgression fragment comprising the BL160 locus, denotes an introgression fragment that is smaller than the introgression fragment as present in *Brassica napus* plant designated BL160, a representative sample of which has been deposited under accession number NCIMB 44185, which still exerts the desired function, i.e. which increases blackleg resistance when present in a *Brassica napus* plant. An assay for determining of whether a functional fragment increases blackleg resistance is provided herein in the Examples section, see Example 3.

As used herein, the terms "introgression", "introgressed" and "introgressing" refer to both a natural and artificial process whereby a genomic fragment of one species, variety or cultivar, termed donor parent, is transduced into the genome of another species, variety or cultivar, termed recipient parent, for example by crossing the donor and recipient parent. The process may optionally be completed by backcrossing the resulting plants to the recipient parent, which is than termed recurrent parent. An introgression fragment is present outside of its natural genomic context, meaning that a plant harbouring an introgression fragment from e.g. *Brassica juncea* is not a *Brassica juncea* plant.

The term "cultivar" (or “cultivated” plant) is used herein to denote a plant having a biological status other than a "wild" status, which "wild" status indicates the original non-cultivated, non-domesticated, or natural state of a plant or accession, and the term cultivated does not include such wild, or weedy plants. The term cultivar does include material with good

agronomic characteristics, such as breeding material, research material, breeding lines, elite breeding lines, synthetic population, hybrid, founder stock/base population, inbred lines, cultivars (open pollinated of hybrid cultivar), segregating population, mutant/genetic stock, and advanced/improved cultivar. The so-called heirloom varieties or cultivars, i.e. open pollinated varieties or cultivars commonly grown during earlier periods in human history and often adapted to specific geographic regions, are in one aspect of the invention encompassed herein as cultivated plants. In one embodiment the term cultivar also includes landraces, i.e. plants (or populations) selected and cultivated locally by humans over many years and adapted to a specific geographic environment and sharing a common gene pool.

"Plant variety" is a group of plants within the same botanical taxon of the lowest grade known, which (irrespective of whether the conditions for the recognition of plant breeder's rights are fulfilled or not) can be defined on the basis of the expression of characteristics that result from a certain genotype or a combination of genotypes, can be distinguished from any other group of plants by the expression of at least one of those characteristics, and can be regarded as an entity, because it can be multiplied without any change. Therefore, the term "plant variety" cannot be used to denote a group of plants, even if they are of the same kind, if they are all characterized by the presence of one locus or gene (or a series of phenotypical characteristics due to this single locus or gene), but which can otherwise differ from one another enormously as regards the other loci or genes.

"Backcrossing" refers to a breeding method by which a (single) trait, such as the blackleg resistance trait of the present invention, can be transferred from one genetic background (also referred to as "donor" generally, but not necessarily, this is an inferior genetic background) into another genetic background (also referred to as "recurrent parent"; generally, but not necessarily, this is a superior genetic background). An offspring of a cross (e.g. an F1 plant obtained by crossing a first plant of a certain plant species comprising the BL160 locus of the present invention with a second plant of the same plant species or of a different plant species that can be crossed with said first plant species wherein said second plant species does not comprise the BL160 locus of the present invention; or an F2 plant or F3 plant, etc., obtained by selfing the F1) is "backcrossed" to a parent plant of said second plant species. After repeated backcrossing, the trait of the donor genetic background, e.g. the BL160 locus conferring the blackleg resistance trait of the present invention, will have been incorporated into the recurrent genetic background. The terms "gene converted" or "conversion plant" or "single locus conversion" in this context refer to plants which are developed by backcrossing wherein essentially all of the desired morphological and/or physiological characteristics of the recurrent parent are recovered in addition to the one or more genes transferred from the donor parent. The plants grown from the seeds produced by backcrossing of the F1 plants with the second parent plant line is referred to as the "BC1 generation". Plants from the BC1 population may be selfed resulting in the BC1F2 generation or backcrossed again with the cultivated parent plant line to provide the BC2 generation. An "M1 population" is a plurality of mutagenized seeds / plants of a certain plant line. "M2, M3, M4, etc." refers to the consecutive generations obtained following selfing of a first mutagenized seed / plant (M1).

A "plant line" or "breeding line" refers to a plant and its progeny. As used herein, the term "inbred line" refers to a plant line which has been repeatedly selfed, preferably more than three time, more preferably more than 6 times.

The terms "progeny", "progenies" and "descendants", as used herein, refer to any and all offspring that are derivable from or obtainable from a plant of the invention that comprises the improved blackleg resistance trait described herein. Progeny may be derived by cell culture or by tissue culture, or by producing seeds of a plant. The term progeny may also encompass plants derived from crossing of at least one resistant parent plant with another plant of the same or another variety or (breeding) line. A progeny is directly derived from, obtained from, obtainable from or derivable from the parent plant by, e.g., traditional breeding methods (selfing and/or crossing) or regeneration or transformation. However, the term "progeny" generally encompasses further generations such as second, third, fourth, fifth, sixth, seventh or more generations, i.e., generations of plants which are derived from, obtained from, obtainable from or derivable from the former generation by, e.g., traditional breeding methods, regeneration or genetic transformation techniques. For example, a second-generation progeny can be produced from a first generation progeny by any of the methods mentioned above.

The BL160 locus as described herein comprises a haplotype that can be characterized by the presence of one or more genetic markers as described herein in more detail. Particularly, the BL160 locus as comprised in the *Brassica napus* plant according to the present invention having enhanced resistance to blackleg is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 6. In one aspect, the introgression fragment further comprises marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 and/or marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7. Particularly, the introgression fragment further comprises marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6 identity to SEQ ID NO: 5 and/or marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7.

In a further aspect, the introgression fragment further does not comprise one or more (i.e. two or more, three or more or all four) of: marker M1 comprising a Cytosine at nucleotide 151 of SEQ ID NO: 1 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 1; marker M2 comprising a Guanine at nucleotide 151 of SEQ ID NO: 2 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 2; marker M3 comprising a

Guanine at nucleotide 151 of SEQ ID NO: 3 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 3; and marker M4 comprising a Guanine at nucleotide 151 of SEQ ID NO: 4 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 4. Particularly, the introgression fragment does not comprise one or more, two or more, three or more, or all of: marker M1 comprising a Cytosine at nucleotide 151 of SEQ ID NO: 1 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 1; marker M2 comprising a Guanine at nucleotide 151 of SEQ ID NO: 2 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 2; marker M3 comprising a Guanine at nucleotide 151 of SEQ ID NO: 3 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 3; and marker M4 comprising a Guanine at nucleotide 151 of SEQ ID NO: 4 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 4. Accordingly, the introgression fragment does not comprise: marker M1; marker M2; marker M3; marker M4; markers M1 and M2; markers M1 and M3; markers M1 and M4; markers M2 and M3; markers M2 and M4; markers M3 and M4; markers M1, M2 and M3; markers M1, M2 and M4; markers M1, M3 and M4; markers M2, M3 and M4; or markers M1, M2, M3 and M4 as further described herein.

In a further aspect, the *Brassica napus* plant according to present invention comprises on chromosome C03 one or more (i.e. two or more, three or more or all four) of: a sequence comprising at least 80% identity to SEQ ID NO: 8; a sequence comprising at least 80% identity to SEQ ID NO: 9; a sequence comprising at least 80% identity to SEQ ID NO: 10; and a sequence comprising at least 80% identity to SEQ ID NO: 11. Particularly, the *Brassica napus* plant according to present invention comprises on chromosome C03 one or more, two or more, three or more, or all of: a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8; a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9; a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10; and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11. Particularly, the Brassica napus plant according to present invention comprises on chromosome C03:
a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8;
a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11;

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11; or

a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 or a sequence having SEQ ID NO: 9, a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 or a sequence having SEQ ID NO: 10 and a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11 or a sequence having SEQ ID NO: 11.

In a further aspect, the *Brassica napus* plant according to present invention comprises on chromosome C03 one or more (i.e. two or more, three or more or all four) of: marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 8; marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 9; marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 10; and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 11. Particularly, the *Brassica napus* plant according to present invention comprises on chromosome C03 one or more, two or more, three or more, or all of: marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID

NO: 8; marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9; marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10; and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11. Particularly, the Brassica napus plant according to present invention comprises on chromosome C03:

marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8;

marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9;

marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10;

marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;

marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8 and marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9;

marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8 and marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10;

marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;

marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 and marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising

at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10;
marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;
marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;
marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 and marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10;
marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;
marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11;
marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9, marker M10 comprising an

Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11; or
marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 9, marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 10 and marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 11.

In a further aspect, the BL160 locus is comprised in a chromosomal segment between a sequence comprising at least 80% identity to SEQ ID NO: 8 and the distal end of chromosome C03. In a further aspect, Particularly, the BL160 locus is comprised in a chromosomal segment between a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 8, or a sequence having SEQ ID NO: 8 and the distal end of chromosome C03. The term “distal end of a chromosome” as used herein refers to the end of the chromosome that is located away from the centromere.

For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e., a position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues. The “optimal alignment” of two sequences is found by aligning the two sequences over the entire length according to the Needleman and Wunsch global alignment algorithm (Needleman and Wunsch, 1970, J Mol Biol 48(3):443-53) in The European Molecular Biology Open Software Suite (EMBOSS, Rice *et al.*, 2000, Trends in Genetics 16(6): 276—277; see e.g. http://www.ebi.ac.uk/emboss/align/index.html) using default settings (gap opening penalty = 10 (for nucleotides) / 10 (for proteins) and gap extension penalty = 0.5 (for nucleotides) / 0.5 (for proteins)). For nucleotides the default scoring matrix used is EDNAFULL and for proteins the default scoring matrix is EBLOSUM62. It will be clear that whenever nucleotide sequences of RNA molecules are defined by reference to nucleotide sequence of corresponding DNA molecules, the thymine (T) in the nucleotide sequence should be replaced by uracil (U). Whether reference is made to RNA or DNA molecules will be clear from the context of the application.

The term “at least 80%” means 80% or more, such as at least 85%, at least 87% at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98% at least 99%, or 100%.

The term “at least 90%” means 90% or more, such as at least 95%, at least 96%, at least 97%, at least 98% or at least 99% or 100%.

“Stringent hybridization conditions” can be used to identify nucleotide sequences, which are substantially identical to a given nucleotide sequence. Stringent conditions are sequence dependent and will be different in different circumstances. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (T_m) for the specific sequences at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically stringent conditions will be chosen in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least 60° C. Lowering the salt concentration and/or increasing the temperature increases stringency. Stringent conditions for RNA-DNA hybridizations (Northern blots using a probe of e.g. 100nt) are for example those which include at least one wash in 0.2X SSC at 63° C for 20min, or equivalent conditions.

“High stringency conditions” can be provided, for example, by hybridization at 65° C in an aqueous solution containing 6x SSC (20x SSC contains 3.0 M NaCl, 0.3 M Na-citrate, pH 7.0), 5x Denhardt's (100X Denhardt's contains 2% Ficoll, 2% Polyvinyl pyrollidone, 2% Bovine Serum Albumin), 0.5% sodium dodecyl sulphate (SDS), and 20 µg/ml denaturated carrier DNA (single-stranded fish sperm DNA, with an average length of 120 - 3000 nucleotides) as non-specific competitor. Following hybridization, high stringency washing may be done in several steps, with a final wash (about 30 min) at the hybridization temperature in 0.2-0.1× SSC, 0.1% SDS.

“Moderate stringency conditions” refers to conditions equivalent to hybridization in the above-described solution but at about 60-62° C. Moderate stringency washing may be done at the hybridization temperature in 1x SSC, 0.1% SDS.

“Low stringency” refers to conditions equivalent to hybridization in the above-described solution at about 50-52° C. Low stringency washing may be done at the hybridization temperature in 2x SSC, 0.1% SDS. See also Sambrook *et al.* (1989) and Sambrook and Russell (2001).

"Average" refers herein to the arithmetic mean.

The BL160 locus according to the present invention thus confers a blackleg resistance phenotype when said BL160 locus is present in the genome of *Brassica napus*. A representative sample of *Brassica napus* seeds comprising BL160 locus as described herein has been deposited and from the deposit, or from descendants of this deposit, the BL160 locus of the present invention can be easily transferred into any other plant that can be crossed with the *Brassica napus* plant, or descendants thereof, grown from the deposited

seeds. Alternatively, other donors can be identified which comprise the same BL160 locus, e.g. comprising the same SNP haplotypes for the BL160 locus, wherein said donor preferably is a *Brassica juncea* plant. In one aspect, the BL160 locus as comprised in the *Brassica napus* plant according to the present invention is as present in, or as obtainable from, or as obtained from, or as comprised in the genome of a *Brassica napus* plant designated BL160, a representative sample of which has been deposited under accession number NCIMB 44185.

In the context of the present invention, it was found that the blackleg resistance phenotype can be observed in *Brassica napus* plants that are heterozygous for the BL160 locus of the present invention and in *Brassica napus* plants that are homozygous for the BL160 locus of the present invention. Based on these results it is concluded that the blackleg resistance effect of the BL160 locus is dominant. In one aspect, accordingly the *Brassica napus* plant according to the present invention is heterozygous for the BL160 locus as described herein. In a further aspect, the *Brassica napus* plant according to the present invention is homozygous for the BL160 locus as described herein. Such a *Brassica napus* plant wherein BL160 locus is present in homozygous form can be easily obtained from a *Brassica napus* plant wherein the BL160 locus is present in heterozygous form using conventional methods, such as by selfing a *Brassica napus* plant wherein the BL160 locus is present in heterozygous form, optionally followed by selecting the offspring comprising the BL160 locus in homozygous form.

As used herein, the term “plant” includes the whole plant or any parts or derivatives thereof, such as plant organs (e.g., harvested or non-harvested seeds, leaves, flowers, etc.), plant cells, plant protoplasts, plant cell or tissue cultures from which whole plants can be regenerated, plant calli, plant cell clumps, and plant cells that are intact in plants, or parts of plants, such as embryos, pollen, ovules, ovaries, fruit pods (e.g., harvested tissues or organs, such as harvested fruit pods or parts thereof), flowers, leaves, seeds, clonally propagated plants, roots, root-stocks, stems, root tips and the like. Also any developmental stage is included, such as seedlings, immature and mature, etc.

“Brassicaceae” or “Brassicaceae plant” as used herein refers to plants belonging to the family of Brassicaceae plants, also called Cruciferae or mustard family. Examples of Brassicaceae are, but are not limited to, *Brassica* species, such as *Brassica napus*, *Brassica oleracea, Brassica rapa, Brassica carinata*, *Brassica nigra*, and *Brassica juncea*; *Raphanus* species, such as *Raphanus caudatus, Raphanus raphanistrum,* and *Raphanus sativus*; *Matthiola* species; *Cheiranthus* species; *Camelina* species, such as *Camelina sativa; Crambe* species, such as *Crambe abyssinica* and *Crambe hispanica*; *Eruca* species, such as *Eruca vesicaria*; *Sinapis* species such as *Sinapis alba*; *Diplotaxis* species; *Lepidium* species; *Nasturtium* species; *Orychophragmus* species; *Armoracia* species, *Eutrema* species; *Lepidium* species; and *Arabidopsis* species.

A “*Brassica* plant” refers to allotetraploid or amphidiploid *Brassica napus* (AACC, 2n=38), *Brassica juncea* (AABB, 2n=36), *Brassica carinata* (BBCC, 2n=34), or to diploid *Brassica rapa* (syn. *B. campestris*) (AA, 2n=20), *Brassica oleracea* (CC, 2n=18) or *Brassica nigra* (BB,

2n=16). In one aspect, the *Brassica napus* plant according to present invention is a *Brassica napus* winter oilseed rape (WOSR) plant or a *Brassica napus* spring oilseed rape (SOSR) plant.

As further described in the Examples, the BL160 locus as described herein was identified by crossing a resistant *Brassica juncea* accession and a *Brassica napus* susceptible line. The *Brassica napus* plant according to the present invention accordingly can be obtained by crossing and subsequent selection, preferably by means of a technical process such as marker-based selection, without that the accordingly obtained plant comprises a technically induced mutation. In one aspect, the *Brassica napus* plant according to the present invention comprises a technically induced mutation. A technically induced mutant, as used herein, is a non-naturally occurring mutant created by man. A technically induced mutant can be produced through mutagenesis. "Mutagenesis" or "induced variation", as used herein, refers to the process in which plant cells (e.g., a plurality of Brassica seeds or other parts, such as pollen, etc.) are subjected to a technique which induces mutations in the DNA of the cells, such as contact with a mutagenic agent, such as a chemical substance (such as ethylmethylsulfonate (EMS), ethylnitrosourea (ENU), etc.) or ionizing radiation (neutrons (such as in fast neutron mutagenesis, etc.), alpha rays, gamma rays (such as that supplied by a Cobalt 60 source), X-rays, UV-radiation, etc.), or a combination of two or more of these. While mutations created by irradiation are often large deletions or other gross lesions such as translocations or complex rearrangements, mutations created by chemical mutagens are often more discrete lesions such as point mutations. For example, EMS alkylates guanine bases, which results in base mispairing: an alkylated guanine will pair with a thymine base, resulting primarily in G/C to A/T transitions. Mutagenesis can comprise random mutagenesis, or can comprise targeted mutagenesis. Mutagenesis can also result in epimutations that cause epigenetic silencing.

Examples of technically induced mutants in *Brassica napus* suitable to the invention mutants in the FATB gene as described in WO2009/007091 or in the FAD3 genes as described in WO2011/060946, or may be podshatter resistant mutant such as mutants described in WO2009/068313 or in WO2010/006732, or mutations conferring herbicide tolerance such as the PM1 and PM2 mutations conferring imidazolinone tolerance (Tan et al., (2005) Pest Management Science 6: 246-257 and US 5545821). Podshatter resistant mutations may be obtainable from seeds having been deposited at the American Type Culture Collection (ATCC, 10801 University Boulevard, Manassas, VA 20110-2209, US) on November 20, 2007, under accession number PTA-8795 or PTA-8796, or at the NCIMB Limited (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, Scotland, AB21 9YA, UK) on July 7, 2008, under accession number NCIMB 41570, NCIMB 41571, NCIMB 41572, NCIMB 41573, NCIMB 41574, or NCIMB 41575. Imidazolinone tolerant mutations may be mutations obtainable from seeds having been deposited at the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A., under Accession No. 40683 or 40684. In one aspect, accordingly, the technically induced mutation as comprised in the *Brassica napus* plant according to the present invention is a podshatter resistant mutation, such as the mutation obtainable from

seeds having been deposited under accession number PTA-8795, PTA-8796, NCIMB 41570, NCIMB 41571, NCIMB 41572, NCIMB 41573, NCIMB 41574, or NCIMB 41575.

In a further aspect, the technically induced mutation as comprised in the *Brassica napus* plant according to the present invention confers herbicide tolerance, such as tolerance to imidazolinone, or wherein said transgene is a gene conferring herbicide tolerance, such as a gene which confers resistance to glufosinate or to glufosinate ammonium or a gene conferring resistance to glyphosate.

The plants according to the invention accordingly may additionally contain an endogenous gene or a transgene, which confers herbicide resistance, such as the bar or pat gene, which confer resistance to glufosinate ammonium (Liberty®, Basta® or Ignite®) (EP 0 242 236 and EP 0 242 246 incorporated by reference); or any modified EPSPS gene, such as the 2mEPSPS gene from maize (EP0 508 909 and EP 0 507 698 incorporated by reference), or glyphosate acetyltransferase, or glyphosate oxidoreductase, which confer resistance to glyphosate (RoundupReady®), or bromoxynitril nitrilase to confer bromoxynitril tolerance, or any modified AHAS gene, which confers tolerance to sulfonylureas, imidazolinones, sulfonylaminocarbonyltriazolinones, triazolopyrimidines or pyrimidyl(oxy/thio)benzoates, such as oilseed rape imidazolinone-tolerant mutants PM1 and PM2, currently marketed as Clearfield® canola. Further, the plants according to the invention may additionally contain an endogenous or a transgene which confers increased oil content or improved oil composition, such as a 12:0 ACP thioesteraseincrease to obtain high laureate, which confers pollination control, such as barnase under control of an anther-specific promoter to obtain male sterility, or barstar under control of an anther-specific promoter to confer restoration of male sterility, or such as the Ogura cytoplasmic male sterility and nuclear restorer of fertility.

Genome editing, also called gene editing, genome engineering, as used herein, refers to the targeted modification of genomic DNA in which the DNA may be inserted, deleted, modified or replaced in the genome. Genome editing may use sequence-specific enzymes (such as endonuclease, nickases, base conversion enzymes) and/or donor nucleic acids (e.g. dsDNA, oligo's) to introduce desired changes in the DNA. Sequence-specific nucleases that can be programmed to recognize specific DNA sequences include meganucleases (MGNs), zinc-finger nucleases (ZFNs), TAL-effector nucleases (TALENs) and RNA-guided or DNA-guided nucleases such as Cas9, Cpf1, CasX, CasY, C2c1, C2c3, certain Argonaut-based systems (see e.g. Osakabe and Osakabe, Plant Cell Physiol. 2015 Mar;56(3):389-400; Ma et al., Mol Plant. 2016 Jul 6;9(7):961-74; Bortesie et al., Plant Biotech J, 2016, 14; Murovec et al., Plant Biotechnol J. 15:917-926, 2017; Nakade et al., Bioengineered Vol 8, No.3:265-273, 2017; Burstein et al., Nature 542, 37–241; Komor et al., Nature 533, 420–424, 2016; all incorporated herein by reference). Donor nucleic acids can be used as a template for repair of the DNA break induced by a sequence specific nuclease. Donor nucleic acids can also be used as such for genome editing without DNA break induction to introduce a desired change into the genomic DNA.

A transgene refers DNA sequences integrated into the genome through transformation.

The gene conferring herbicide resistance may be the bar or pat gene, which confer resistance to glufosinate ammonium (Liberty®, Basta® or Ignite®) [EP 0 242 236 and EP 0 242 246 incorporated by reference]; or any modified EPSPS gene, such as the 2mEPSPS gene from maize [EP0 508 909 and EP 0 507 698 incorporated by reference], or glyphosate acetyltransferase, or glyphosate oxidoreductase, which confer resistance to glyphosate (RoundupReady®), or bromoxynitril nitrilase to confer bromoxynitril tolerance.
The plants according to the invention which additionally contain a gene which confers resistance to glufosinate ammonium (Liberty®, Basta® or Ignite®) may contain a gene coding for a phosphinothricin-N-acetyltransferase (PAT) enzyme, such as a coding sequence of the bialaphos resistance gene (bar) of Streptomyces hygroscopicus. Such plants may, for example, comprise the elite event RF-BN1 as described in WO01/41558.
The plants according to the invention which contain a gene which confers resistance to glyphosate (RoundupReady®) may contain a glyphosate resistant EPSPS, such as a CP4 EPSPS, or an N-acetyltransferase (gat) gene. Such plants may, for example, comprise the elite event RT73 as described in WO02/36831, or elite event MON88302 as described in WO11/153186, or event DP-073496-4 as described in WO2012/071040.

The term “transformation” as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell. Transformation of plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., (1982) Nature 296, 72-74; Negrutiu I et al. (1987) Plant Mol Biol 8: 363-373); electroporation of protoplasts (Shillito R.D. et al. (1985) Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A et al., (1986) Mol. Gen Genet 202: 179-185); DNA or RNA-coated particle bombardment (Klein TM et al., (1987) Nature 327: 70) infection with (non-integrative) viruses and the like. Transgenic plants are preferably produced via *Agrobacterium*-mediated transformation. The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. After introduction, the plant may be selected for the presence of the BL160 locus of the present invention.

The present invention also relates to a method for protecting cultivated plants in a field, wherein said plants comprise i) the BL160 locus of the present invention, and ii) at least one resistance gene conferring herbicide tolerance and wherein said method comprises applying the said herbicide to the cultivated plants in order to control weeds. In an embodiment, said herbicide is glufosinate. In another embodiment, said herbicide is glufosinate ammonium. In another embodiment, said herbicide is glyphosate. The present invention accordingly provides a method for the protection of a group of cultivated plants according to the present

invention comprising a technically induced mutation which confers herbicide tolerance in a field wherein weeds are controlled by the application of a composition comprising one or more herbicidal active ingredients. In one aspect, the herbicide used in the method for the protection of a group of cultivated plants according to the present invention is glufosinate or glufosinate ammonium or glyphosate.

The plants and seeds according to the invention may be further treated with a chemical compound, such as a chemical compound selected from the following lists: Herbicides: Clethodim, Clopyralid, Diclofop, Ethametsulfuron, Fluazifop, Glufosinate, Glyphosate, Metazachlor, Quinmerac, Quizalofop, Tepraloxydim, Trifluralin. Fungicides / PGRs: Azoxystrobin, N-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (Benzovindiflupyr, Benzodiflupyr), Bixafen, Boscalid, Carbendazim, Carboxin, Chlormequat-chloride, Coniothryrium minitans, Cyproconazole, Cyprodinil, Difenoconazole, Dimethomorph, Dimoxystrobin, Epoxiconazole, Famoxadone, Fluazinam, Fludioxonil, Fluopicolide, Fluopyram, Fluoxastrobin, Fluquinconazole, Flusilazole, Fluthianil, Flutriafol, Fluxapyroxad, Iprodione, Isopyrazam, Mefenoxam, Mepiquat-chloride, Metalaxyl, Metconazole, Metominostrobin, Paclobutrazole, Penflufen, Penthiopyrad, Picoxystrobin, Prochloraz, Prothioconazole, Pyraclostrobin, Sedaxane, Tebuconazole, Tetraconazole, Thiophanate-methyl, Thiram, Triadimenol, Trifloxystrobin, Bacillus firmus, Bacillus firmus strain I-1582, Bacillus subtilis, Bacillus subtilis strain GB03, Bacillus subtilis strain QST 713, Bacillus pumulis, Bacillus. pumulis strain GB34.

Insecticides: Acetamiprid, Aldicarb, Azadirachtin, Carbofuran, Chlorantraniliprole (Rynaxypyr), Clothianidin, Cyantraniliprole (Cyazypyr), (beta-)Cyfluthrin, gamma-Cyhalothrin, lambda-Cyhalothrin, Cypermethrin, Deltamethrin, Dimethoate, Dinetofuran, Ethiprole, Flonicamid, Flubendiamide, Fluensulfone, Fluopyram,Flupyradifurone, tau-Fluvalinate, Imicyafos, Imidacloprid, Metaflumizone, Methiocarb, Pymetrozine, Pyrifluquinazon, Spinetoram, Spinosad, Spirotetramate, Sulfoxaflor, Thiacloprid, Thiamethoxam, 1-(3-chloropyridin-2-yl)-N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-3-{[5-(trifluoromethyl)-2H-tetrazol-2-yl]methyl}-1H-pyrazole-5-carboxamide, 1-(3-chloropyridin-2-yl)-N-[4-cyano-2-methyl-6-(methylcarbamoyl)phenyl]-3-{[5-(trifluoromethyl)-1H-tetrazol-1-yl]methyl}-1H-pyrazole-5-carboxamide, 1-{2-fluoro-4-methyl-5-[(2,2,2-trifluorethyl)sulfinyl]phenyl}-3-(trifluoromethyl)-1H-1,2,4-triazol-5-amine, (1E)-N-[(6-chloropyridin-3-yl)methyl]-N'-cyano-N-(2,2-difluoroethyl)ethanimidamide, Bacillus firmus, Bacillus firmus strain I-1582, Bacillus subtilis, Bacillus subtilis strain GB03, Bacillus subtilis strain QST 713, Metarhizium anisopliae F52.

The present invention further provides hybrid plants, which may have advantages such as improved uniformity, vitality and/or disease tolerance. In one aspect the *Brassica napus* plant according to the present invention is a F1 hybrid, more preferably a single cross F1 hybrid plant. The present invention further provides a haploid plant or dihaploid plant derived from the *Brassica napus* plant as provided by the present invention. In one aspect, accordingly, the present invention provides haploid plants and/or dihaploid (double haploid) plants of plant of

the invention are encompassed herein, which comprise the BL160 locus as described herein. Haploid and dihaploid plants can for example be produced by anther or microspore culture and regeneration into a whole plant. For dihaploid production chromosome doubling may be induced using known methods, such as colchicine treatment or the like. So, in one aspect a *Brassica napus* plant is provided, comprising the BL160 locus as described herein, wherein the plant is a dihaploid plant. In one aspect, the *Brassica napus* plant according to the present invention is an inbred plant. Such an inbred plant is highly homozygous, for instance by repeated selfing crossing steps. Such an inbred plant may be very useful as a parental plant for the production of F1 hybrid seed.

The terms "F1, F2, etc." refer to the consecutive related generations following a cross between two parent plants or parent lines. The plants grown from the seeds produced by crossing two plants or lines is called the F1 generation. Selfing the F1 plants results in the F2 generation, etc. The term "hybrid" plant (or hybrid seed) refers to a plant or seed obtained from crossing two inbred parent lines. The term "F1 hybrid" plant (or "F1 hybrid" seed or "F1 seed") refers to a first-generation plant or seed obtained from crossing two inbred parent lines.

"Vegetative propagation" or "clonal propagation" refers to propagation of plants from vegetative tissue, e.g. by propagating plants from cuttings or by in vitro propagation. In vitro propagation involves in vitro cell or tissue culture and regeneration of a whole plant from the in vitro culture. Clones (i.e. genetically identical vegetative propagations) of the original plant can thus be generated by in vitro culture. "Cell culture" or "tissue culture" refers to the in vitro culture of cells or tissues of a plant. "Regeneration" refers to the development of a plant from cell culture or tissue culture or vegetative propagation. “Non-propagating cell” refers to a cell which cannot be regenerated into a whole plant.

The present invention further provides a seed produced by the *Brassica napus* plant according to the present invention, wherein the seed comprises the BL160 locus as described herein.

The present invention further provides a seed from which the *Brassica napus* plant according to the present invention can be grown.

Furthermore, the invention provides a plurality of seed according to the present invention. A seed of the invention can be distinguished from other seeds due to the presence of the BL160 locus as described herein, either phenotypically (based on the blackleg resistance phenotype of the present invention) and/or using molecular methods to detect the presence of the BL160 locus in the cells or tissues, such as molecular genotyping methods to detect BL160 locus of the present invention or sequencing. Seeds include for example seeds produced by a plant of the invention which is heterozygous for the BL160 locus after self-pollination and optionally selection of those seeds which comprise one or two copies of the BL160 locus (e.g. by non-destructive seed sampling methods and analysis of the presence of the BL160 locus, or seed produced after cross-pollination, e.g. pollination of a plant of the invention with pollen from

another Brassica plant, preferably from another *Brassica napus* plant, or pollination of another *Brassica napus* plant with pollen of a plant of the invention).

The present invention further provides seeds obtained from the methods of producing plants as described herein.

In one aspect, a plurality of seed is packaged into a container (e.g. a bag, a carton, a can etc.). Containers may be any size. The seeds may be pelleted prior to packing (to form pills or pellets) and/or treated with various compounds, including seed coatings.

In a further aspect a plant part, obtained from (obtainable from) a plant of the invention is provided herein, and a container or a package comprising said plant part. The present invention accordingly further provides a plant cell, tissue or plant part of the *Brassica napus* plant according to present invention or of the seed according to the present invention, comprising the BL160 locus as described herein.

In a further aspect, the plant part is a plant cell. In still a further aspect, the plant part is a non-regenerable cell or a regenerable cell. In another aspect the plant cell is a somatic cell. A non-regenerable cell is a cell which cannot be regenerated into a whole plant through in vitro culture. The non-regenerable cell may be in a plant or plant part (e.g. leaves) of the invention. The non-regenerable cell may be a cell in a seed, or in the seed-coat of said seed. Mature plant organs, including a mature leaf, a mature stem or a mature root, contain at least one non-regenerable cell.

In a further aspect the plant cell is a reproductive cell, such as an ovule or a cell which is part of a pollen. In an aspect, the pollen cell is the vegetative (non-reproductive) cell, or the sperm cell (Tiezzi, Electron Microsc. Review, 1991). Such a reproductive cell is haploid. When it is regenerated into whole a plant, it comprises the haploid genome of the starting plant. If chromosome doubling occurs (e.g. through chemical treatment), a double haploid plant can be regenerated. In one aspect the plant of the invention comprising the BL160 locus as described herein is a haploid or a double haploid *Brassica napus* plant according to the present invention.

Moreover, there is provided an in vitro cell culture or tissue culture of the *Brassica napus* plant of the invention in which the cell- or tissue culture is derived from a plant part described herein, such as, for example and without limitation, a leaf, a pollen, an embryo, cotyledon, hypocotyls, callus, a root, a root tip, an anther, a flower, a seed or a stem, or a part of any of them, or a meristematic cell, a somatic cell, or a reproductive cell.

The present invention further provides a vegetatively propagated plant, wherein said plant is propagated from a plant part according to the present invention.

Further, isolated cells, in vitro cell cultures and tissue cultures, protoplast cultures, plant parts, harvested material (e.g. harvested rape seeds), pollen, ovaries, flowers, seeds, stamen,

flower parts, etc. comprising in each cell at least one copy of the BL160 locus as described herein are provided. Thus, when said cells or tissues are regenerated or grown into a whole *Brassica napus* plant, the plant comprises the BL160 locus capable of conferring a blackleg resistance phenotype.

Thus, also an in vitro cell culture and/or tissue culture of cells or tissues of plants of the invention is provided. The cell or tissue culture can be treated with shooting and/or rooting media to regenerate a *Brassica napus* plant.

Also vegetative or clonal propagation of plants according to the invention is encompassed herein. Many different vegetative propagation techniques exist. Cuttings (nodes, shoot tips, stems, etc.) can for example be used for in vitro culture as described above. Also other vegetative propagation techniques exist and can be sued, such as grafting, or air layering. In air layering a piece of stem is allowed to develop roots while it is still attached to the parent plant and once enough roots have developed the clonal plant is separated from the parent.

Thus, in one aspect a method is provided comprising:
(a) obtaining a part of a plant of the invention (e.g. cells or tissues, e.g. cuttings),
(b) vegetatively propagating said plant part to generate an identical plant from the plant part.

Thus, also the use of vegetative plant parts of plants of the invention for clonal/vegetative propagation is an aspect of the invention. In one aspect a method is provided for vegetatively reproducing a *Brassica napus* plant of the invention comprising the BL160 locus as described herein is provided. Also a vegetatively produced *Brassica napus* plant comprising the BL160 locus as described herein is provided.

In another aspect a *Brassica napus* plant according to the invention, comprising the BL160 locus as described herein, is propagated by somatic embryogenesis techniques.

Also provided is a *Brassica napus* plant regenerated from any of the above-described plant parts, or regenerated from the above-described cell or tissue cultures, said regenerated plant comprising in its genome the BL160 locus as described herein. This plant can also be referred to as a vegetative propagation of plants of the invention.

The invention also relates to a food or feed product comprising or consisting of a plant part described herein.

In one aspect plants, plant parts and cells according to the present invention are obtained by a technical method such as a marker assisted selection method as described herein. In one aspect plants, plant parts and cells according to the present invention are not exclusively obtained by means of an essentially biological process, e.g. as defined by Rule 28(2) EPC. In one aspect, accordingly, the present invention provides the *Brassica napus* plant as further described herein, wherein said plant comprises a technically induced mutation, such as a

randomly induced mutation, a targeted gene modification or a transgene. The technically induced mutation may result in the BL160 blackleg resistance locus according to the present invention or may be any other technically induced mutation. In one aspect, a process for the production of plants or animals is essentially biological if it consists entirely of natural phenomena such as crossing or selection e.g. as defined by Rule 26(5) EPC and Article 2(2) of the Biotech Directive 98/44/EC.

The term "exclusively" in the context of the proviso that a plant or plant part is not exclusively obtained by means of an essentially biological process is used herein to mean that a plant or plant part originating from a technical process or characterised by a technical intervention in the genome is not covered by the exclusion from patentability even if in addition an essentially biological process (such as crossing and selection) is applied in its production or propagation. Accordingly, the progeny of a plant or a plant part according to the present invention comprising at least one copy of the BL160 locus as described herein does not fall outside the scope of the claims merely because exclusively an essentially biological process was used to obtain said progeny.

In a further embodiment, a blackleg resistant *Brassica napus* plant or plant part according to the invention is provided, comprising the BL160 blackleg resistance locus according to the invention, and at least one other disease resistance gene, said other disease resistance gene selected from the group consisting of a clubroot resistance gene, a further blackleg resistance gene, a Sclerotinia resistance gene, a Verticillium resistance gene, a Fusarium wilt resistance gene, a Root Rot resistance gene, an Aster Yellows resistance gene, an Alternaria resistance gene, a Grey Stem resistance gene, and a Turnip yellows virus resistance gene.

Said clubroot resistance gene and/or resistance QTL may be a *Crr2, Crr4, Crr3, CRk, CRc, CR2a, CR2b, pb-3, pb-4, Pb-Bo1, Pb-Bo2, Pb-Bo3, Pb-Bo4, Pb-Bo5a, Pb-Bo5b, Pb-Bo8, Pb-Bo9a, Pb-Bo9b, Pb-Bn1, PbBn-01:60-1, PbBn-01:60-2, PbBn-01:60-3, PbBn-01:60-4, PbBn-01:07-1, PbBn-01:07-2, PbBn-01:07-3, , PbBn-e4x04-1, PbBn-a-1, PbBn-l-1, PbBn-l-2, PbBn-k-1, PbBn-k-2. PbBn-k-3, PbBn-Korp-1, PbBn-Korp-2, PbBn-Korp-3, PbBn-Korp-4, PbBn-Korp-5* as described by Piao (Piao et al., 2009, J Plant Growth Regul 28: 252), or may be a CRa gene as described by Ueno et al. (Ueno et al., 2012, Plant Mol Biol 80: 621), a Crr1 gene as described by Hatakeyama et al., (Hatakeyama et al., 2013, PLOS one 8: e54745) and in WO2012/039445, a *CR*b gene as described by Kato et al., (Kato et al., 2013, Breeding Science 63: 116), (herein incorporated by reference), or may be *CRb, CRc, CRd, CRk, CrrA5, CRs, Rcr1, Rcr2, Rcr4, Rcr8, Rcr9* (Hejna et al., 2019, Mol Breed. 39(8): 112), *Rcr3, Rcr5, Rcr9*^{wa} (Karim et al., 2020, Int J Mol Sci. 21(14): 5033). Moreover, said clubroot resistance gene may be a CLR1 or CLR2 gene as described in WO 2017/102923 A1 (herein incorporated by reference).

Said further blackleg resistance gene may, for example, be BLMR1 and BLMR2 (WO 2011/044694), LepR3 (Larkan et al., 2013, New Phytol 197:595 and WO 2008/101343), or Lem-08-syl (EP 1547462 and US 2005/0142122). Moreover, said further blackleg resistance gene may be Rlm1, Rlm2, Rlm3, Rlm4, Rlm5, Rlm6, Rlm7, Rlm8, Rlm9, Rlm10, Rlm11, RlmJ1,

RlmS, LepR1, LepR2, LepR4, LmJR1 or LmJR2 (Larkan et al., 2016, supra), Rlm12 (Raman et al., or Rlm13 (Raman et al., 2021, Front. Plant Sci. 12:654604).

A Sclerotinia resistance gene may be a sclerotinia resistance gene as described in WO 2005/090578.

Said other disease resistance gene (or disease resistance genes) may be present in their native chromosomal position. For example, said other disease resistance genes can be introduced by introgression in the plant according to the invention from the cultivar or -species from which they are derived.

Hybrid seeds of the plants according to the invention may be generated by crossing two inbred parental lines, wherein one of the inbred parental lines comprises the BL160 locus according to the invention. In order to produce pure hybrid seeds one of the parental lines is male sterile and is pollinated with pollen of the other line. By growing parental lines in rows and only harvesting the F1 seed of the male sterile parent, pure hybrid seeds are produced. To generate male sterile parental lines, the system as described in EP 0,344,029 or US 6,509,516 may be used, wherein a gene encoding a phytotoxic protein (barnase) is expressed under the control of a tapetum specific promoter, such as TA29, ensuring selective destruction of tapetum cells. Transformation of plants with the chimeric gene pTA29:barnase results in plants in which pollen formation is completely prevented /Mariani *et al.* (1990), *Nature* 347: 737-741). Cytochemical and histochemical analysis of anther development of *Brassica napus* plants comprising the chimeric pTA29-barnase gene is described by De Block and De Brouwer ((1993), *Planta* 189:218-225). To restore fertility in the progeny of a male-sterile plant the male-sterile plant (MS parent) is crossed with a transgenic plant (RF parent) carrying a fertility-restorer gene, which when expressed is capable of inhibiting or preventing the activity of the male-sterility gene (U.S. Pat. Nos. 5,689,041; 5,792,929; De Block and De Brouwer, supra). The use of co-regulating genes in the production of male-sterile plants to increase the frequency of transformants having good agronomical performance is described in WO96/26283. Typically, when the sterility DNA encodes a barnase, the co-regulating DNA will encode a barstar, preferably an optimized barstar gene is used as described in published PCT patent application WO 98/10081. It is understood that different promoters may be used to drive barnase expression in order to render the plant male sterile. Likewise, barstar may be operably linked to different promoters, such as 35S from Cauliflower mosaic virus.

Male sterile plants can also be generated using other techniques, such as cytoplasmic male sterility/restorer systems [e.g. the Ogura system, published US patent application 20020032916, US 6,229,072, WO97/02737, US 5,789,566 or the Polima system of US 6,365,798, WO98/54340 or the Kosena system of WO95/09910, US 5,644,066].

Either the MS parent or the RF parent, or both, may comprise the BL160 locus according to the invention. This can be accomplished by either introducing the BL160 locus into an elite *B. napus* line and then transforming this line with pTA29-barnase or with pNOS-barstar using known methods. Alternatively the BL160 blackleg resistance genes can be introduced directly

into a transgenic MS or RF parent line, by crossing a plant comprising the BL160 locus with the MS parent or RF-parent, or by transformation of the MS parent or the RF parent. The F1 hybrid seeds generated from the cross between the MS and RF parent will then contain the BL160 locus as described herein.

The present invention further provides methods wherein a *Brassica napus* plant as described herein comprising the BL160 locus of the present invention is used and/or obtained.

The present invention further provides a method for identifying and/or selecting a *Brassica napus* plant or plant part comprising determining whether said plant or plant part comprises in its genome the BL160 locus as described herein. Accordingly, the present invention further provides a method for identifying and/or selecting a *Brassica napus* plant or plant part comprising determining whether said plant or plant part comprises in its genome the BL160 locus, wherein said BL160 locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6.

In one aspect the method for identifying and/or selecting a *Brassica napus* plant or plant part as described herein comprises the step of identifying at least one marker within 10 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7. Particularly, the method for identifying and/or selecting a *Brassica napus* plant or plant part as described herein comprises the step of identifying at least one marker within 10 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7.

A “molecular marker”, or a “marker”, as used herein, refers to a polymorphic locus, i.e. a polymorphic nucleotide (a so-called single nucleotide polymorphism or SNP) or a polymorphic DNA sequence (which can be insertion of deletion of a specific DNA sequence at a specific locus, or polymorphic DNA sequences). A marker refers to a measurable, genetic characteristic with a fixed position in the genome, which is normally inherited in a Mendelian fashion, and which can be used for mapping of a trait of interest. Thus, a molecular marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change, i.e. a single nucleotide polymorphism or SNP, or a long DNA sequence, such as microsatellites or Simple Sequence Repeats (SSRs). The nature of the marker is dependent on the molecular analysis used and can be detected at the DNA, RNA or protein level. Genetic mapping can be

performed using molecular markers such as, but not limited to, RFLP (restriction fragment length polymorphisms; Botstein *et al.* (1980), Am J Hum Genet 32:314-331; Tanksley *et al.* (1989), Bio/Technology 7:257-263), RAPD [random amplified polymorphic DNA; Williams *et al.* (1990), NAR 18:6531-6535], AFLP [Amplified Fragment Length Polymorphism; Vos *et al.* (1995) *NAR* 23:4407-4414]. Appropriate primers or probes are dictated by the mapping method used.

The term “marker allele" refers to the version of the marker that is present in a particular plant at one of the chromosomes. Typically, a marker can exist as or can be said to have or to comprise two marker alleles. The term “haplotype”, as used herein, refers to a specific combination of marker alleles as present within a certain plant or group of (related) plants. As described herein, a marker allele can be the version of the marker that is present in the resistant line (BL160 blackleg resistance marker allele). The version of the same marker that is present in the susceptible line can be referred to as BL160 blackleg susceptibility marker allele.

The term “AFLP®” (AFLP® is a registered trademark of KeyGene N.V., Wageningen, The Netherlands), “AFLP analysis” and “AFLP marker” is used according to standard terminology (Vos *et al.* (1995), *NAR* 23:4407-4414; EP0534858). Briefly, AFLP analysis is a DNA fingerprinting technique which detects multiple DNA restriction fragments by means of PCR amplification. The AFLP technology usually comprises the following steps: (i) the restriction of the DNA with two restriction enzymes, preferably a hexa-cutter and a tetra-cutter, such as EcoRI, PstI and MseI; (ii) the ligation of double-stranded adapters to the ends of the restriction fragments, such as EcoRI, PstI and MseI adaptors; (iii) the amplification of a subset of the restriction fragments using two primers complementary to the adapter and restriction site sequences, and extended at their 3' ends by one to three “selective” nucleotides, i.e., the selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. AFLP primers thus have a specific sequence and each AFLP primer has a specific code; (iv) gel electrophoresis of the amplified restriction fragments on denaturing slab gels or capilaries; (v) the visualization of the DNA fingerprints by means of autoradiography, phosphor-imaging, or other methods. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. An AFLP marker, as used herein, is a DNA fragment of a specific size, which is generated and visualized as a band on a gel by carrying out an AFLP analysis. Each AFLP marker is designated by the primer combination used to amplify it, followed by the approximate size (in base pairs) of the amplified DNA fragment. It is understood that the size of these fragments may vary slightly depending on laboratory conditions and equipment used. Every time reference is made herein to an AFLP marker by referring to a primer combination and the specific size of a fragment, it is to be understood that such size is approximate, and comprises or is intended to include the slight variations observed in different labs. Each AFLP marker represents a certain locus in the genome.

The term "SSR" refers to Simple Sequence Repeats or microsatellite [Tautz *et al.* (1989), *NAR* 17:6463-6471]. Short Simple Sequence stretches occur as highly repetitive elements in all eukaryotic genomes. Simple sequence loci usually show extensive length polymorphisms. These simple sequence length polymorphisms (SSLP) can be detected by polymerase chain reaction (PCR) analysis and be used for identity testing, population studies, linkage analysis and genome mapping.

It is understood that molecular markers can be converted into other types of molecular markers. When referring to a specific molecular marker in the present invention, it is understood that the definition encompasses other types of molecular markers used to detect the genetic variation originally identified by the specific molecular markers. For example, if an AFLP marker is converted into another molecular marker using known methods, this other marker is included in the definition. For example, AFLP markers can be converted into sequence-specific markers such as, but not limited to STS (sequenced-tagged-site) or SCAR (sequence-characterized-amplified-region) markers using standard technology as described in Meksem *et al.* [(2001), *Mol Gen Genomics* 265(2):207-214], Negi *et al.* [(2000), *TAG* 101:146-152], Barret *et al.* (1989), *TAG* 97:828-833], Xu *et al.* [(2001), *Genome* 44(1):63-70], Dussel *et al.* [(2002), *TAG* 105:1190-1195] or Guo *et al.* [(2003), *TAG* 103:1011-1017]. For example, Dussel *et al.* [(2002), *TAG* 105:1190-1195] converted AFLP markers linked to resistance into PCR-based sequence tagged site markers such as indel (insertion/deletion) markers and CAPS (cleaved amplified polymorphic sequence) markers.

Suitable molecular markers are, for example SNP markers (Single Nucleotide Polymorphisms), AFLP markers, microsatellites, minisatellites, Random Amplified Polymorphic DNA's (RAPD) markers, RFLP markers, Sequence Characterized Amplified Regions (SCAR) markers, and others, such as TRAP markers described by Hu *et al.* 2007, Genet Resour Crop Evol 54: 1667-1674).

Methods and assays for marker detection, or for analyzing the genomic DNA for the presence of a marker, are widely known in the art. The presence of a marker can, for example be detected in hybridization-based methods (e.g. allele-specific hybridization), using Taqman, Invader, PCR-based methods, oligonucleotide ligation based methods, or sequencing-based methods.

A useful assay for detection of SNP markers is for example KBioscience Competitive Allele - Specific PCR. For developing the KASP-assay 70 base pairs upstream and 70 basepairs downstream of the SNP are selected and two allele-specific forward primers and one allele specific reverse primer is designed. See e.g. Allen et al. 2011, Plant Biotechnology J. 9, 1086-1099, especially p1097-1098 for KASP assay method (incorporated herein by reference).

A "molecular marker linked to the BL160 blackleg resistance locus", or a "molecular marker linked to the presence of the BL160 blackleg resistance locus" as used herein refers to a molecular marker in a region in the genome that inherits with the BL160 blackleg resistance locus as a single genetic unit in at least 50% of the cases. Thus, in this respect, the term

linked can be a separation of about 50 cM, or less such as about 40 cM, about 30 cM, about 20 cM, about 10 cM, about 7.5 cM, about 6 cM, about 5 cM, about 4 cM, about 3 cM, about 2.5 cM, about 2 cM, or even less.

A "molecular marker linked to the BL160 blackleg resistance locus", or a "molecular marker linked to the presence of the BL160 blackleg resistance locus" can also be a marker located in the introgression fragment from *Brassica juncea* chromosome B08 as further described herein.

Suitable are markers that are linked to the BL160 blackleg resistance locus can be developed using methods known in the art. New markers suitable for the invention can be developed based on the BL160 sequence. It is understood that such markers can be developed by comparing the sequence of the BL160 blackleg resistance locus from the resistant Brassicaceae line with the sequence of the same locus in a susceptible Brassicaceae line; identifying a specific sequence region in the BL160 blackleg resistance locus which does not occur in the corresponding locus of the susceptible Brassicaceae line. A molecular marker linked to the BL160 blackleg resistance locus can thus be a marker detecting the presence of the BL160 blackleg resistance locus. A molecular marker linked to the BL160 blackleg resistance locus can also be a marker in the sequences flanking the BL160 blackleg resistance locus, which is polymorphic between lines comprising the BL160 blackleg resistance locus and lines lacking, but which inherits with the BL160 blackleg resistance locus as a single genetic unit in at least 50% of the cases.

Markers suitable to determine the presence of the BL160 blackleg resistance locus can be the markers that are linked to BL160 blackleg resistance locus.

The absence of the BL160 blackleg resistance locus can be determined by the absence of marker alleles that are linked to the presence of the BL160 blackleg resistance locus (BL160 blackleg resistance marker alleles). Furthermore, markers suitable to determine the absence of the BL160 blackleg resistance locus can be marker alleles which are linked to the BL160 blackleg susceptibility locus (BL160 blackleg susceptibility marker alleles).

Identification of PCR products specific for the BL160 blackleg resistance locus and for the corresponding locus in the susceptible Brassicaceae line can occur e.g. by size estimation after gel or capillary electrophoresis (e.g. for the BL160 blackleg resistance locus and for the corresponding locus in the susceptible Brassicaceae line comprising a number of inserted or deleted nucleotides which results in a size difference between the fragments amplified from the BL160 blackleg resistance locus and for the corresponding locus in the susceptible Brassicaceae, such that said fragments can be visibly separated on a gel); by evaluating the presence or absence of the two different fragments after gel or capillary electrophoresis, whereby the diagnostic PCR amplification of the BL160 blackleg resistance locus can, optionally, be performed separately from the diagnostic PCR amplification of the corresponding locus in the susceptible line; by direct sequencing of the amplified fragments; or by fluorescence-based detection methods.

A further embodiment provides methods to determine the presence or absence of a BL160 blackleg resistance locus in a biological sample, comprising providing genomic DNA from said biological sample, and analyzing said DNA for the presence of at least one molecular marker, wherein the at least one molecular marker is linked to the presence or absence of the BL160 blackleg resistance locus, wherein said BL160 blackleg resistance locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 6. In one aspect the method to determine the presence or absence of a BL160 blackleg resistance locus in a biological sample as described herein comprises the step of identifying at least one marker within 10 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7.

In yet a further embodiment, a kit is provided for the detection of the BL160 blackleg resistance locus according to the invention in Brassicaceae DNA samples, wherein said kit comprises at least one primer or probe which specifically recognizes a molecular marker linked to said BL160 blackleg resistance locus as provided by the present invention.

In particular, the methods and kits according to the invention are suitable to determine the presence of the BL160 blackleg resistance locus. The presence of the BL160 blackleg resistance locus can be determined using at least one molecular marker, wherein said one molecular marker is linked to the presence of the BL160 blackleg resistance locus as defined herein.

A “biological sample” can be a plant or part of a plant such as a plant tissue or a plant cell.

“Providing genomic DNA” as used herein refers to providing a sample comprising genomic DNA from the plant.

The sample can refer to a tissue sample which has been obtained from said plant, such as, for example, a leaf sample, comprising genomic DNA from said plant. The sample can further refer to genomic DNA which is obtained from a tissue sample, such as genomic DNA which has been obtained from a tissue, such as a leaf sample. Providing genomic DNA can include, but does not need to include, purification of genomic DNA from the tissue sample. Providing genomic DNA thus also includes obtaining tissue material from a plant or larger piece of tissue and preparing a crude extract or lysate therefrom.

A “kit”, as used herein, refers to a set of reagents for the purpose of performing the method of the invention, more particularly, the identification of the BL160 blackleg resistance locus in biological samples.

In an embodiment, the kit comprises at least one oligonucleotide for identifying the BL160 blackleg resistance locus of the present invention. The at least oligonucleotide shall specifically bind to a BL160 blackleg resistance locus of the invention.

In a preferred embodiment of the kit of the invention comprises at least two specific primers (i.e. oligonucleotides) for identification of the BL160 blackleg resistance locus. The primers shall allow for specifically amplifying the BL160 blackleg resistance marker alleles as set forth herein (such as SEQ ID NOs: 9-14, particularly SEQ ID NOs: 11-12). Suitable primer pairs for a KASP assay are shown, for example, in Table 3 in the Examples section.

Optionally, the kit can further comprise any other reagent. Alternatively, according to another embodiment of this invention, the kit can comprise at least one specific probe, which specifically hybridizes with nucleic acid of biological samples to identify the presence of the BL160 blackleg resistance locus therein.

Optionally, the kit can further comprise any other reagent (such as but not limited to hybridizing buffer, label) for identification of the BL160 blackleg resistance locus in biological samples, using the specific probe.

The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g., purity of seed lots), detection of the presence or absence of the BL160 blackleg resistance locus in plant material or material comprising or derived from plant material, such as but not limited to food or feed products. The zygosity status of the BL160 blackleg resistance locus can be determined by using alternative sets of primers and/or probes that are specific for the BL160 locus and the corresponding locus in a susceptible Brassicaceae line.

The term “primer” or “oligonucleotide” as used herein encompasses any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process, such as PCR. Typically, primers are oligonucleotides from 10 to 30 nucleotides (such as 15 to 30 nucleotides), but longer sequences can be employed. Primers may be provided in double-stranded form, though the single-stranded form is preferred. Probes can be used as primers, but are designed to bind to the target DNA or RNA and need not be used in an amplification process. Typically, the probe or primer shall be capable of detecting the BL160 locus of the present invention (e.g. by binding to it).

The term “recognizing” as used herein when referring to specific primers, refers to the fact that the specific primers specifically hybridize to a specific nucleic acid sequence under suitable conditions, such as the conditions set forth in the method (such as the conditions of

the PCR identification protocol), whereby the specificity is determined by the presence of positive and negative controls.

The present invention further provides the use of a genetic marker specific of the BL160 locus as described herein for selecting a *Brassica napus* plant having an enhanced blackleg resistance phenotype.

The present invention further provides a method for producing a *Brassica napus* plant having a blackleg resistance phenotype, said method comprising the step(s) of: (i) crossing a first *Brassica napus* plant and a second plant, wherein the first Brassica napus plant comprises in its genome the BL160 locus as described herein; (ii) optionally harvesting seed from the crossing of (i) and selecting seed comprising the BL160 locus in its genome.

Also suitable is a method for producing a *Brassica napus* plant having a blackleg resistance phenotype, said method comprising the step(s) of: (i) providing a seed mixture harvested from a cross between a first *Brassica napus* plant and a second plant, wherein the first *Brassica napus* plant comprises in its genome the BL160 locus as described herein; and (ii) selecting seed comprising the BL160 locus in its genome.

In one aspect, the method for producing a *Brassica napus* plant having a blackleg resistance phenotype as described herein further comprises the step of selecting seed comprising the BL160 locus in its genome by identifying at least one marker within 10 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7. Particularly, the method for producing a *Brassica napus* plant having a blackleg resistance phenotype according to the present invention further comprises the step of selecting seed comprising the BL160 locus in its genome by identifying at least one marker within 10 cM, 9 cM, 8 cM, 7 cM, 6 cM, 5 cM, 4 cM, 3 cM, 2 cM or 1 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to SEQ ID NO: 7

The plants according to the present invention accordingly can be used in a conventional breeding scheme to produce more plants with the same characteristics or to introduce the characteristic of the presence of the BL160 blackleg resistance locus according to the invention in other varieties of the same or related plant species, or in hybrid plants. The obtained plants can further be used for creating propagating material. Plants according to the invention can further be used to produce gametes, seeds (including crushed seeds and seed cakes), seed oil, embryos, either zygotic or somatic, progeny or hybrids of plants obtained by

methods of the invention. Seeds obtained from the plants according to the invention are also encompassed by the invention.

"Creating propagating material", as used herein, relates to any means know in the art to produce further plants, plant parts or seeds and includes inter alia vegetative reproduction methods (e.g. air or ground layering, division, (bud) grafting, micropropagation, stolons or runners, storage organs such as bulbs, corms, tubers and rhizomes, striking or cutting, twin-scaling), sexual reproduction (crossing with another plant) and asexual reproduction (e.g. apomixis, somatic hybridization).

The present invention further provides a method for enhancing the blackleg resistance phenotype of a *Brassica napus* plant, said method comprising introgressing the BL160 locus as described herein into said *Brassica napus* plant.

Suitable to the invention is a method to produce blackleg free *Brassica napus* plants, comprising the steps of sowing seeds from the *Brassica napus* plants according to the invention comprising a BL160 blackleg resistance locus, growing the plants in the field, optionally spraying the plants with fungicides, and harvesting.

Also provided is a method of producing food, feed, or an industrial product, comprising obtaining the plant according to the invention or a part thereof; and preparing the food, feed or industrial product from the plant or part thereof. In a further object, said food or feed is oil, meal, grain, starch, flour or protein; or said industrial product is biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical. In a preferred embodiment, the food is an oil.

A further object provides the use of the plants according to the invention to produce oilseed rape oil or an oilseed rape seed cake, or a seed, or a crop of oilseed rape.

The present invention further provides the use of the BL160 locus as described herein for enhancing the blackleg resistance phenotype in a *Brassica napus* plant.

The present invention further provides a method for stacking the BL160 locus and a further gene of interest, said method comprising introducing said other gene of interest in the genome of the *Brassica napus* plant according to the present invention at a genomic position which is genetically linked to the BL160 locus.

A representative sample of seeds of a *Brassica napus* plant comprising blackleg resistance locus BL160 according to the present invention were deposited by BASF Agricultural Solutions Seed US LLC on 14 July 2023 at the NCIMB Ltd. (Wellheads Place, Aberdeen, Dyce, AB21 7GB Scotland) according to the Budapest Treaty, under the Expert Solution (EPC 2000, Rule 32(1)). Seeds were given the following deposit number: NCIMB 44185 (Brassica napus BL160).

Representative samples of the *Leptosphaeria maculans* isolates were deposited by BASF Agricultural Solutions Seed US LLC on 1 March 2023 at the IDAC. (1015 Arlington Street, Winnipeg, Manitoba Canada R3E 3R2) according to the Budapest Treaty, under the Expert Solution. The different *Leptosphaeria maculans* isolates were given the following deposit number: IDAC 010323-01 (15SK40); IDAC 010323-02 (18SK100); IDAC 010323-03 (19SK07); and IDAC 010323-04 (19SK21).

The Applicant requests that samples of the biological material and any material derived therefrom be only released to a designated Expert in accordance with Rule 32(1) EPC or related legislation of countries or treaties having similar rules and regulation, until the mention of the grant of the patent, or for 20 years from the date of filing if the application is refused, withdrawn or deemed to be withdrawn.

Access to the deposit will be available during the pendency of this application to persons determined by the Director of the U.S. Patent Office to be entitled thereto upon request. Subject to 37 C.F.R. § 1.808(b), all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent. The deposit will be maintained 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 it ever becomes nonviable during that period. Applicant does not waive any rights granted under this patent on this application or under the Plant Variety Protection Act (7 USC 2321 et seq.).

It is to be understood that this invention is not limited to the particular methodology or protocols. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. It must be noted that as used herein and in the appended claims, the singular forms "a," "and," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "a vector" is a reference to one or more vectors and includes equivalents thereof known to those skilled in the art, and so forth. The term "about" is used herein to mean approximately, roughly, around, or in the region of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 20 percent, preferably 10 percent up or down (higher or lower). As used herein, the word "or" means any one member of a particular list and also includes any combination of members of that list. The words "comprise," "comprising," "include," "including," and "includes" when used in this specification and in the following claims are intended to specify the presence of one or more stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more other features, integers, components, steps, or groups thereof.

Sequences

The sequence listing contained in the file named "230200WO01_SEQLISTING_St26.xml", which is 58 kilobytes (size as measured in Microsoft Windows®), contains 44 sequences SEQ ID NO: 1 through SEQ ID NO: 44 is filed herewith by electronic submission and is incorporated by reference herein.

In the description and examples, reference is made to the following sequences:
SEQ ID NO: 1: genetic marker M1
SEQ ID NO: 2: genetic marker M2
SEQ ID NO: 3: genetic marker M3
SEQ ID NO: 4: genetic marker M4
SEQ ID NO: 5: genetic marker M5
SEQ ID NO: 6: genetic marker M6
SEQ ID NO: 7: genetic marker M7
SEQ ID NO: 8: genetic marker M8
SEQ ID NO: 9: genetic marker M9
SEQ ID NO: 10: genetic marker M10
SEQ ID NO: 11: genetic marker M11
SEQ ID NO: 12: Assay M1: Primer 1
SEQ ID NO: 13: Assay M1: Primer 2
SEQ ID NO: 14: Assay M1: Common primer
SEQ ID NO: 15: Assay M2: Primer 1
SEQ ID NO: 16: Assay M2: Primer 2
SEQ ID NO: 17: Assay M2: Common primer
SEQ ID NO: 18: Assay M3: Primer 1
SEQ ID NO: 19: Assay M3: Primer 2
SEQ ID NO: 20: Assay M3: Common primer
SEQ ID NO: 21: Assay M4: Primer 1
SEQ ID NO: 22: Assay M4: Primer 2
SEQ ID NO: 23: Assay M4: Common primer
SEQ ID NO: 24: Assay M5: Primer 1
SEQ ID NO: 25: Assay M5: Primer 2
SEQ ID NO: 26: Assay M5: Common primer
SEQ ID NO: 27: Assay M6: Primer 1
SEQ ID NO: 28: Assay M6: Primer 2
SEQ ID NO: 29: Assay M6: Common primer
SEQ ID NO: 30: Assay M7: Primer 1
SEQ ID NO: 31: Assay M7: Primer 2
SEQ ID NO: 32: Assay M7: Common primer
SEQ ID NO: 33: Assay M8: Primer 1
SEQ ID NO: 34: Assay M8: Primer 2
SEQ ID NO: 35: Assay M8: Common primer
SEQ ID NO: 36: Assay M9: Primer 1

SEQ ID NO: 37: Assay M9: Primer 2
SEQ ID NO: 38: Assay M9: Common primer
SEQ ID NO: 39: Assay M10: Primer 1
SEQ ID NO: 40: Assay M10: Primer 2
SEQ ID NO: 41: Assay M10: Common primer
SEQ ID NO: 42: Assay M11: Primer 1
SEQ ID NO: 43: Assay M11: Primer 2
SEQ ID NO: 44: Assay M11: Common primer

Examples

Unless stated otherwise in the Examples, all recombinant techniques are carried out according to standard protocols as described in "Sambrook J and Russell DW (eds.) (2001) Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, New York" and in "Ausubel FA, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (eds.) (2006) Current Protocols in Molecular Biology. John Wiley & Sons, New York".

Standard materials and references are described in "Croy RDD (ed.) (1993) Plant Molecular Biology LabFax, BIOS Scientific Publishers Ltd., Oxford and Blackwell Scientific Publications, Oxford" and in "Brown TA, (1998) Molecular Biology LabFax, 2nd Edition, Academic Press, San Diego". Standard materials and methods for polymerase chain reactions (PCR) can be found in "McPherson MJ and Møller SG (2000) PCR (The Basics), BIOS Scientific Publishers Ltd., Oxford" and in "PCR Applications Manual, 3rd Edition (2006), Roche Diagnostics GmbH, Mannheim or www.roche-applied-science.com".

It should be understood that a number of parameters in any lab protocol such as the PCR protocols in the below Examples may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA or the selection of other primers in a PCR method may dictate other optimal conditions for the PCR protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals.

Example 1 – BL160 introgression from *B. juncea* into *B. napus*

A resistant *Brassica juncea* accession and a *Brassica napus* spring susceptible line (Recurrent Parent, RP) against a *Leptosphaeria maculans* isolate (Lm452-1) were selected to produce F1 of interspecific cross.

F1 obtained from *B. napus* x *B. juncea* cross was used as pollinator to cross onto *B. napus* RP line to produce BC1F1 seeds.

A set of 680 BC1F1 seedlings were inoculated using the differential *Leptosphaeria maculans* isolate Lm452-1 and phenotyped for their resistance. The same plants were also genotyped, using SNP's in the B subgenome of *B. juncea*. MTA analyses identified that the resistance is associated to a B08 region. The locus conferring the resistance to *L. maculans* originating from *B. juncea* and introgressed into *B. napus* was named BL160.

A resistant BC1F1 plant with smallest B08 fragment (including markers M5, M6 and M7) was selected for BC1F2 generation. 268 BC1F2 progenies were phenotyped for their resistance to the isolate Lm452-1 of the pathogen and genotyped (A, B and C subgenomes). In this population, it was shown that the BL160 locus was genetically linked to chromosome C03 of *B. napus* (top part of the chromosome C03, using Darmor version 10 as a reference, Rousseau-Gueutin et al. 2020).

Phenotyping with the differential isolate was carried out for the subsequent selfing generations (BC1F2, BC1F3, BC1F4). The introgressed B08 fragment remained stable in the different generations.

Example 2 – Phenotypic characterization

Single spore isolates from Canada were used to inoculate plants from different seedlots from the BC1F5 and BC1F6 generations, along with *Rlm6* carrier lines to differentiate resistance of BL160 vs. *Rlm6*.

BC1F6 seed lots CN21Q10000360 & CN21Q10000363 derived from Resistant BC1F5 plants carrying the BL160 locus and *Rlm6* carrier lines were phenotyped with different *L. maculans* isolates to compare their resistance profile.

L maculans isolates used in the context of the present invention were collected from infested canola stubble from the field in Saskatchewan, Canada in 2015, 2018 and 2019. Small plant tissues were excised from plant tissues, surface sterilized in 5% bleach and cultured on V-8 Bacto Agar. Pure single spore isolates were obtained by sub-culturing diluted spores on potato dextrose agar (PDA). Individual spores were then excised along with a small piece of PDA and sub-cultured back on V-8 Bacto Agar. Single spore cultures where then allocated the names to designate the collection year, province, and a sequential number at the end.: Particularly, the following *Leptosphaeria maculans* isolates have been used in the context of the present invention: *Leptosphaeria maculans* isolate designated as 15SK40, a representative sample of which has been deposited under Accession Number IDAC 010323-01; *Leptosphaeria maculans* isolate designated as 18SK100, a representative sample of which has been deposited under Accession Number IDAC 010323-02; *Leptosphaeria maculans* isolate designated as 19SK07, a representative sample of which has been deposited under Accession Number IDAC 010323-03; and *Leptosphaeria maculans* isolate designated as 19SK21, and a representative sample of which has been deposited under Accession Number IDAC 010323-04.

Seeds were planted in 36-cell flats targeting 16 seedlings per each seed lot. Inoculation was done 7 days after seeding on cotyledons using a spore susception of 10^7 spores per mL by placing 10uL of the spore suspension on each wounded cotyledon, resulting in a total of 4 inoculation points per plant or 64 inoculation points per seed lot. Disease ratings were conducted 14 days after inoculation using a 0-9 scale, where: 0-5 = R and 6-9 = S.

Table 1: Phenotyping results of different plants carrying/not carrying BL160 or *Rlm6*, using different *L. maculans* isolates (18SK100, 19SK07, 19SK21 and 15SK40). Material 9 was not tested.

Accession	Generation	*R* gene /locus	18SK100	19SK07	19SK21	15SK40	Accession Description
Material 1	Inbred	*Rlm6* fixed	R	S	R	R	*B. napus* (WOSR int. *Rlm6*)
Material 2	Inbred	*Rlm6* fixed	R	S	R	R	*B. napus* (SOSR int. *Rlm6*)
Material 3	Inbred	*Rlm6* fixed	R	S	R	R	*B. napus* (SOSR int. *Rlm6*)
Material 4	Inbred	*Rlm6* fixed	R	S	R	R	*B. napus* (SOSR int. *Rlm6*)
Material 5	Inbred	*Rlm6* fixed	R	S	R	R	*B. napus* (SOSR int. *Rlm6*)
Material 6	Inbred	BL160 + other unknown	R	R	R	R	*B. juncea* BL160 donor
Material 7	BC1F6	BL160 fixed	R	S	S	R	*B. napus*, derived from a R BC1F5 plant homozygous for BL160
Material 8	BC1F6	BL160 segregating	11R/5S	S	S	12R/4S	*B. napus*, derived from a R BC1F5 plant heterozygous for BL160
Topas DH16516	Inbred	None	S	S	S	S	*B. napus* (SOSR susceptible check)
Westar	Inbred	None	S	S	S	S	*B. napus* (SOSR susceptible check)
Material 9							Recurrent parent

Table 2: Phenotyping results of CN21Q10000360 plants carrying BL160 and GT Cougar carrying *Rlm6*, using different *L. maculans* isolates. R: resistant; S: Susceptible.

Isolate	GT Cougar Rlm6	CN21Q10000360 BL160
Aus562	S	S
CR7-15	R	R
09SK09a	R	R
12CC-111	S	R
12CC-115	R	R
13SK39a	R	R
13SK43a	S	S
13SK45a	R	R
15MB13	R	R
15MB33	R	R
15SK40	R	R
15SK41	R	R
15SK44	R	R
17SK06	R	R
17SK72	R	R
15SK53	R	R
19SK07	S	S
13CH69	R	R
18MB30	R	R
18MB33	R	R
19SK06	R	R
21AB07	S	R
21AB119	R	R
21AB120	R	R

Table 3: Phenotyping results of plants carrying BL160 and plants (Topas and Westar) not carrying a blackleg resistance gene. Rating is given on a scale 0-9, where 0-9 scale, where: 0-5 = Resistant (R) and 6-9 = Susceptible (S)/

Isolate	R-gene	Name/Pl #	Avg rating	Class
18MB30	BL160	Plant 1	1.00	R
	BL160	Plant 2	1.00	R
	BL160	Plant 3	1.75	R
	BL160	Plant 4	1.00	R
	-	Topas	9.00	S
	-	Topas	9.00	S
	-	Westar	9.00	S
	-	Westar	9.00	S
18MB33	BL160	Plant 1	1.00	R
	BL160	Plant 2	1.00	R
	BL160	Plant 3	1.00	R
	BL160	Plant 4	1.00	R
	-	Topas	9.00	S
	-	Topas	9.00	S
	-	Westar	7.00	S
	-	Westar	9.00	S
19SK06	BL160	Plant 1	1.00	R
	BL160	Plant 2	1.00	R
	BL160	Plant 3	1.00	R
	BL160	Plant 4	1.00	R
	-	Topas	9.00	S
	-	Topas	9.00	S
	-	Westar	9.00	S
	-	Westar	9.00	S
15CH69	BL160	Plant 1	1.00	R
	BL160	Plant 2	1.00	R
	BL160	Plant 3	1.00	R
	BL160	Plant 4	1.00	R
	-	Topas	9.00	S
	-	Topas	9.00	S

	-	Westar	9.00	S
	-	Westar	9.00	S
21AB120	BL160	Plant 1	1.00	R
	BL160	Plant 2	1.00	R
	BL160	Plant 3	1.00	R
	BL160	Plant 4	1.00	R
	-	Topas	9.00	S
	-	Topas	9.00	S
	-	Westar	9.00	S
	-	Westar	9.00	S

Table 1 shows that *B. napus* carrying *Rlm*6 showed a resistance response to the *L. maculans* isolates 18SK100, 19SK21 and 15SK40, and a susceptible response to isolate 19SK07.

B. juncea donor line is carrying the BL160 locus and other unknown resistance gene(s). This line showed a resistance response to all the 4 differential isolates 18SK100, 19SK07, 19SK21 and 15SK40.

B. napus carrying the BL160 locus showed resistance responses to 18SK100 and 15SK40 compared to isolates 19SK07 and 19SK21 where it showed susceptible reactions.

Several other blackleg isolates were tested. *B. napus* carrying the BL160 locus showed resistance responses to many different isolates (see, Tables 2 and 3). Importantly, *B. napus* carrying BL160 showed resistance to isolates 12CC-111 and 21AB07, whereas *B. napus* carrying *Rlm*6 showed susceptible reactions to these isolates (Table 2).

The segregation for the resistance observed in the BC1F6 plants (Table 1; Material 8) derived from a resistant BC1F5 plant indicates a dominant effect of BL160.

Example 3 – Genotypic characterization

Different BL160 and *Rlm*6 carriers were genotyped on chromosome B08 and C03 using markers M1 to M11. Markers M1 to M7 were used to characterize the B08 fragment in the different carriers. Markers M8 to M11 were used to characterize the *B. napus* deletion on chromosome C03.

The primers as shown in the herein below provided Table 4 were used to detect for the presence of any one of markers M1 to M11 in a Kompetitive allele specific PCR (KASP) Assay.

Table 4: Primers used in KASP Assay.

M1	Primer 1	GAAGGTGACCAAGTTCATGCTGCAATGAGTTGTATGTCTTTGCCGA (SEQ ID NO:12)
	Primer 2	GAAGGTCGGAGTCAACGGATTCAATGAGTTGTATGTCTTTGCCGC (SEQ ID NO:13)
	Common primer	GGATGAGATGTCTCGAGTTGTATTCTTAA (SEQ ID NO:14)
M2	Primer 1	GAAGGTGACCAAGTTCATGCTAATAAGCCGTTTTTTACATGCAATGGAAA (SEQ ID NO:15)
	Primer 2	GAAGGTCGGAGTCAACGGATTAAGCCGTTTTTTACATGCAATGGAAG (SEQ ID NO:16)
	Common primer	GTGCTTGTATCACCTAAGGAGGCAT (SEQ ID NO:17)
M3	Primer 1	GAAGGTGACCAAGTTCATGCTGTCTCCACTCTCCACTACTGATTTA (SEQ ID NO:18)
	Primer 2	GAAGGTCGGAGTCAACGGATTCTCCACTCTCCACTACTGATTTG (SEQ ID NO:19)
	Common primer	TCAGCMAAACTCCATGTACTGGTTTAG (SEQ ID NO:20)
M4	Primer 1	GAAGGTGACCAAGTTCATGCTAATGCCTTGCTCGAAGCATTGACA (SEQ ID NO:21)
	Primer 2	GAAGGTCGGAGTCAACGGATTGCCTTGCTCGAAGCATTGACG (SEQ ID NO:22)
	Common primer	GCTTCTTACAACGAWCCTCCTAACG (SEQ ID NO:23)
M5	Primer 1	GAAGGTGACCAAGTTCATGCTGAGGATGATGTCTCTTTTCTCCATAAA (SEQ ID NO:24)
	Primer 2	GAAGGTCGGAGTCAACGGATTAGGATGATGTCTCTTTTCTCCATAAC (SEQ ID NO:25)
	Common primer	CTGCCCTTCACATGCGCTCCTT (SEQ ID NO:26)
M6	Primer 1	GAAGGTGACCAAGTTCATGCTGGAGTTGGCTGTTTTCCTGTGTATT (SEQ ID NO:27)
	Primer 2	GAAGGTCGGAGTCAACGGATTGAGTTGGCTGTTTTCCTGTGTATG (SEQ ID NO:28)
	Common primer	GTCACTCTTACAACGTTTATAACCTTCTTA (SEQ ID NO:29)
M7	Primer 1	GAAGGTGACCAAGTTCATGCTACACCTAGTTTACGTTCGAT (SEQ ID NO:30)
	Primer 2	GAAGGTCGGAGTCAACGGATTACACCTAGTTTACGTTCGAG (SEQ ID NO:31)
	Common primer	TCATTTGGAAACTTGTCTTTCC (SEQ ID NO:32)
M8	Primer 1	GAAGGTGACCAAGTTCATGCTGTGGTCTCTTGCATCTATCCATACT (SEQ ID NO:33)
	Primer 2	GAAGGTCGGAGTCAACGGATTGGTCTCTTGCATCTATCCATACC (SEQ ID NO:34)
	Common primer	GGAGGCTTTGTTTATGTTCATGACGATAA (SEQ ID NO:35)
M9	Primer 1	GAAGGTGACCAAGTTCATGCTAATMTGAGAGCGGTGGCTA (SEQ ID NO:36)
	Primer 2	GAAGGTCGGAGTCAACGGATTAATMTGAGAGCGGTGGCTC (SEQ ID NO:37)
	Common primer	GAGTGAGTGACACTGGATCATA (SEQ ID NO:38)
M10	Primer 1	GAAGGTGACCAAGTTCATGCTCTGATTCAACTTTGACCGAGCT (SEQ ID NO:39)

	Primer 2	GAAGGTCGGAGTCAACGGATTCTGATTCAACTTTGACCGAGCC (SEQ ID NO:40)
	Common primer	AAGAAAASCAGTGAAAAGTATAGCT (SEQ ID NO:41)
M11	Primer 1	GAAGGTGACCAAGTTCATGCTTGAKAGTGAGACGGTAATCTTCCTG (SEQ ID NO:42)
	Primer 2	GAAGGTCGGAGTCAACGGATTTGAKAGTGAGACGGTAATCTTCCTA (SEQ ID NO:43)
	Common primer	CAAGAAATGAAATGGACAACGGA (SEQ ID NO:44)

Table 5: Genotyping results of different blackleg resistant donors, carrying either the BL160 locus (BL160 *B. juncea* donor and BL160 introgressed into *B. napus*), or *Rlm6* (Darmor-Rlm6 and GT-Cougar). The markers used are mapped on chromosome B08 (Sichuan-Yellow genome v1 (*Brassica juncea*), Kang et al. 2021) and C03 (Darmor-bzh genome v10 (*Brassica napus*), Rousseau-Gueutin et al. 2020).

marker identifier	**chr Sichuan-Yellow genome (v1)**	**phys.pos (bp) Sichuan-Yellow genome (v1)**	**chr Darmor genome (v10)**	**phys.pos (bp) Darmor genome (v10)**	**BL160 *B. juncea* donor**	**BL160 introgressed into *B. napus* (BC1F7)**	**Material 10 (*B. napus* *Rlm6* source)**	**Darmor-*Rlm6* (*B. napus* INRA *Rlm6* source)**	**GT-Cougar (*B. napus* *Rlm6* source)**
M1	B08	57,258,389			CC	--	--	--	--
M2	B08	58,638,691			GG	--	--	--	GG
M3	B08	65,982,835			GG	--	--	AA	GG
M4	B08	67,652,796			GG	--	AA	GG	GG
M5	B08	68,284,468			GG	GG	GG	GG	GG
M6	B08	69,699,585			GG	GG	TT	TT	TT
M7	B08	71,165,503			AA	AA	AA	AA	AA
M8			C03	3,503,807	--	AA	--	--	--
M9			C03	4,762,650	--	CC	AA	--	--
M10			C03	7,581,828	--	AA	GG	GG	--
M11			C03	14,752,425	--	TT	CC	TT	TT

The different resistant *B. juncea* and *B. napus* plants genotyped are characterized by a B08 fragment of variable size. All resistant plants are carrying a common fragment including markers M5, M6 and M7. The plant Material 10 showed an additional call for marker M4. The plant Darmor-*Rlm*6 showed 2 extra calls, for markers M3 and M4. The plant GT-Cougar showed 3 extra calls, for markers M2, M3 and M4.

The marker M6 is differentiating the plants carrying the BL160 locus (allele GG) from the 3 other resistant plants (allele TT).

These *B. napus* plants were also characterized by different deletions on the chromosome C03. For Material 10, the deletion included marker M8. For Darmor-*Rlm*6, the deletion included both markers M8 and M9. For GT-Cougar, the deletion was spanning markers M8, M9 and M10.

Claims

1. A *Brassica napus* plant having enhanced resistance to blackleg, wherein said resistance is provided by blackleg resistance locus BL160 (BL160 locus), wherein said BL160 locus is comprised in an introgression fragment from *Brassica juncea* chromosome B08, wherein said introgression fragment comprises marker M6 comprising a Guanine at nucleotide 151 of SEQ ID NO: 6 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 6.

2. The *Brassica napus* plant of claim 1, wherein the introgression fragment further comprises marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 and/or marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7.

3. The *Brassica napus* plant of claim 1 or 2, wherein the introgression fragment does not comprise one or more of:
marker M1 comprising a Cytosine at nucleotide 151 of SEQ ID NO: 1 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 1;
marker M2 comprising a Guanine at nucleotide 151 of SEQ ID NO: 2 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 2;
marker M3 comprising a Guanine at nucleotide 151 of SEQ ID NO: 3 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 3; and
marker M4 comprising a Guanine at nucleotide 151 of SEQ ID NO: 4 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 4.

4. The *Brassica napus* plant according to any one of claims 1 to 3, wherein said plant comprises on chromosome C03 one or more of:
a sequence comprising at least 80% identity to SEQ ID NO: 8;
a sequence comprising at least 80% identity to SEQ ID NO: 9;
a sequence comprising at least 80% identity to SEQ ID NO: 10; and
a sequence comprising at least 80% identity to SEQ ID NO: 11.

5. The *Brassica napus* plant according to any one of claims 1 to 4, wherein said plant comprises on chromosome C03 one or more of:
marker M8 comprising an Adenine at nucleotide 61 of SEQ ID NO: 8 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 8;
marker M9 comprising a Cytosine at nucleotide 61 of SEQ ID NO: 9 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 9;
marker M10 comprising an Adenine at nucleotide 61 of SEQ ID NO: 10 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 10; and
marker M11 comprising a Thymine at nucleotide 61 of SEQ ID NO: 11 or at nucleotide 61 of a sequence comprising at least 80% identity to SEQ ID NO: 11.

6. The *Brassica napus* plant according to any one of claims 1 to 5, wherein said BL160 locus is comprised in a chromosomal segment between a sequence comprising at least 80% identity to SEQ ID NO: 8 and the distal end of chromosome C03.

7. The *Brassica napus* plant according to any one of claims 1 to 6, wherein the BL160 locus is as present in, or as obtainable from, or as obtained from, or as comprised in the genome of a *Brassica napus* plant designated BL160, a representative sample of which has been deposited under accession number NCIMB 44185.

8. The *Brassica napus* plant according to any one of claims 1 to 7, wherein the BL160 locus confers resistance to *Leptosphaeria maculans* isolate designated 18SK100, a representative sample of which has been deposited under accession number IDAC 010323-02 and/or confers resistance to *Leptosphaeria maculans* isolate designated 15SK40, a representative sample of which has been deposited under accession number IDAC 010323-01.

9. The *Brassica napus* plant of any one of claims 1 to 8, wherein the BL160 locus confers resistance to *Leptosphaeria maculans* isolate designated 12CC-111 and 21AB07.

10. The *Brassica napus* plant according to any one of claims 1 to 9, wherein said plant is heterozygous for the BL160 locus.

11. The *Brassica napus* plant according to any one of claims 1 to 9, wherein said plant is homozygous for the BL160 locus.

12. The *Brassica napus* plant according to any one of claims 1 to 11, wherein said plant is a *Brassica napus* WOSR plant or a *Brassica napus* SOSR plant.

13. The *Brassica napus* plant according to any one of claims 1 to 12, wherein said plant comprises a technically induced mutation, preferably a modification in the genome created with genome editing technologies or a transgene.

14. The *Brassica napus* plant according to claim 13, wherein the technically induced mutation confers herbicide tolerance, such as tolerance to imidazolinone, or wherein said transgene is a gene conferring herbicide tolerance, such as a gene which confers resistance to glufosinate or to glufosinate ammonium or a gene conferring resistance to glyphosate.

15. The *Brassica napus* plant according to claim 13 or 14, wherein the technically induced mutation is a podshatter resistant mutation, such as the mutation obtainable from seeds having been deposited under accession number PTA-8795, PTA-8796, NCIMB 41570, NCIMB 41571, NCIMB 41572, NCIMB 41573, NCIMB 41574, or NCIMB 41575.

16. The *Brassica napus* plant according to any one of claims 1 to 15, wherein said plant is a F1 hybrid.

17. Seed produced by the *Brassica napus* plant according to any of the preceding claims, wherein the seed comprises the BL160 locus as defined in any one of claims 1 to 8.

18. A seed from which the *Brassica napus* plant according to any one of claims 1 to 16 can be grown.

19. A plant cell, tissue or plant part of the *Brassica napus* plant according to any one of claims 1 to 16 or of the seed according to claim 17 or 18, comprising the BL160 locus as defined in any one of claims 1 to 8.

20. A haploid plant or dihaploid plant derived from the *Brassica napus* plant according to any one of claims 1 to 16.

21. A method for identifying and/or selecting a *Brassica napus* plant or plant part comprising determining whether said plant or plant part comprises in its genome the BL160 locus as defined in any one of claims 1 to 8.

22. The method according to claim 21, which method comprises the step of identifying at least one marker within 10 cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7.

23. A method for producing a *Brassica napus* plant having a blackleg resistance phenotype, said method comprising the step(s) of:
    (i) crossing a first *Brassica napus* plant and a second plant, wherein the first *Brassica napus* plant comprises in its genome the BL160 locus as defined in any one of claims 1 to 8;
    (ii) optionally harvesting seed from the crossing of (i) and selecting seed comprising the BL160 locus in its genome.

24. A method for producing a *Brassica napus* plant having a blackleg resistance phenotype, said method comprising the step(s) of:
    (i) providing a seed mixture harvested from a crossing between a first *Brassica napus* plant and a second plant, wherein the first *Brassica napus* plant comprises in its genome the BL160 locus as defined in any one of claims 1 to 8;
    (ii) selecting seed comprising the BL160 locus in its genome.

25. The method according to claim 23 or 24, further comprising the step of selecting seed comprising the BL160 locus in its genome by identifying at least one marker within 10

cM of the marker interval from marker M5 comprising a Guanine at nucleotide 151 of SEQ ID NO: 5 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 5 to marker M7 comprising an Adenine at nucleotide 151 of SEQ ID NO: 7 or at nucleotide 151 of a sequence comprising at least 80% identity to SEQ ID NO: 7.

26. Method for enhancing the blackleg resistance phenotype of a *Brassica napus* plant, said method comprising introgressing the BL160 locus as defined in any one of claims 1 to 8 into said *Brassica napus* plant.

27. Use of the BL160 locus as defined in any one of claims 1 to 8 for enhancing the blackleg resistance phenotype in a *Brassica napus* plant.

28. Use of a genetic marker specific of the BL160 locus as defined in any one of claims 1 to 8 for selecting a *Brassica napus* plant having an enhanced blackleg resistance phenotype.

29. A method for the protection of a group of cultivated plants according to claim 14 in a field wherein weeds are controlled by the application of a composition comprising one or more herbicidal active ingredients.

30. The method according to claim 29, wherein the herbicide is glufosinate or glufosinate ammonium or glyphosate.

31. A method for stacking the BL160 locus and a further gene of interest, said method comprising introducing said other gene of interest in the genome of the *Brassica napus* plant according to any one of claims 1 to 12 at a genomic position which is genetically linked to the BL160 locus.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/079410

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01H1/04 A01H5/10 A01H6/20
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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEVRE A M ET AL: "Selection of stable Brassica napus-B. juncea recombinant lines resistant to blackleg (Leptosphaeria maculans)", THEOR.APPL. GENET., vol. 95, 1 November 1997 (1997-11-01), pages 1104-1111, XP093239866, Retrieved from the Internet: URL:https://link.springer.com/content/pdf/10.1007/s001220050669.pdf> abstract page 1108, column 1 page 1110, column 1, paragraph 2 - paragraph 3	1-31

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 January 2025	27/01/2025

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	Krüger, Julia

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/079410

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/079410

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RASHID M. HARUNUR ET AL: "Development of molecular markers linked to the Leptosphaeria maculans resistance gene Rlm6 and inheritanceof SCAR and CAPS markers in Brassica napus 3 Brassicajuncea interspecific hybrids", PLANT BREEDING, vol. 137, no. 3, 7 May 2018 (2018-05-07), pages 402-411, XP093238953, DE ISSN: 0179-9541, DOI: 10.1111/pbr.12587 Retrieved from the Internet: URL:https://onlinelibrary.wiley.com/doi/full-xml/10.1111/pbr.12587> cited in the application page 403, column 1, paragraph 4 - column 2, paragraph 1 page 405, column 2, paragraph 2 page 406, column 2 -----	1-31
A	INTURRISI FABIAN ET AL: "In silico integration of disease resistance QTL, genes and markers with the Brassica juncea physical map", MOLECULAR BREEDING, SPRINGER NETHERLANDS, DORDRECHT, vol. 42, no. 7, 27 June 2022 (2022-06-27), XP037879196, ISSN: 1380-3743, DOI: 10.1007/S11032-022-01309-5 [retrieved on 2022-06-27] page 6, column 1; table 1 -----	1-31
A	EP 1 547 462 B1 (BAYER BIOSCIENCE NV [BE]) 7 April 2010 (2010-04-07) paragraphs [0003], [0020], [0032], [0036]; example 1 -----	1-31

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/079410

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1547462 B1	07-04-2010	AT E463157 T1	15-04-2010
		AU 2003271381 A1	14-07-2005
		CA 2451589 A1	24-06-2005
		CN 1653883 A	17-08-2005
		DK 1547462 T3	19-07-2010
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		PL 1547462 T3	30-09-2010
		PT 1547462 E	12-07-2010
		SI 1547462 T1	31-08-2010
		US 2005142122 A1	30-06-2005