



(43) International Publication Date
19 October 2017 (19.10.2017)

(51) International Patent Classification:
C12N 15/82 (2006.01)

(21) International Application Number:
PCT/EP2017/058388

(22) International Filing Date:
7 April 2017 (07.04.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
EP16165100.5 13 April 2016 (13.04.2016) EP

(71) Applicant: BAYER CROPSCIENCE NV [BE/BE]; J.E. Mommaertslaan 14, 1831 Diegem (BE).

(72) Inventors: DENOLF, Peter; Schonenberg 6, 9620 Velzeke (BE). VAN AUDENHOVE, Katrien; Kiekenstraat 10, 9000 Gent (BE). TESKE, John; Technologiepark 38, 9052 Gent (BE).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA,

MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to the identity of the inventor (Rule 4.17(i))
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: SEED- AND FUNICULUS-PREFERENTIAL PROMOTERS AND USES THEREOF

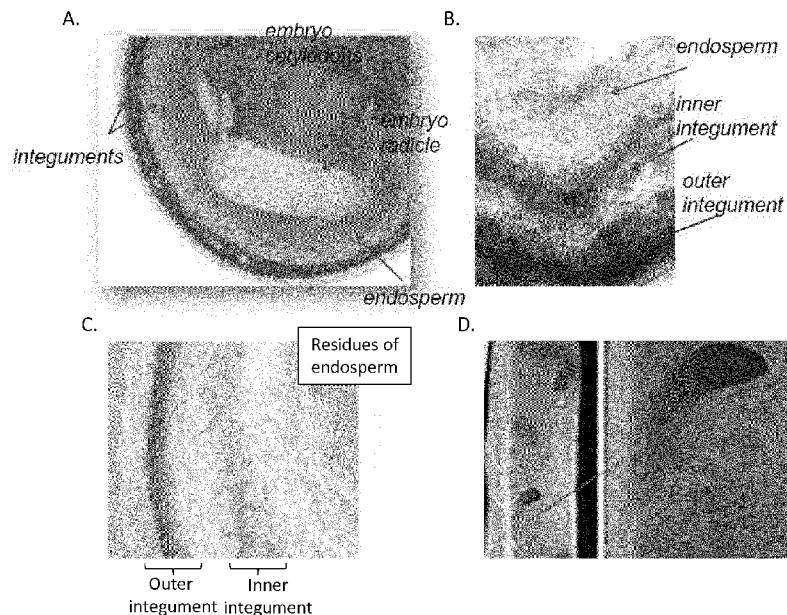


Figure 3

(57) Abstract: The present invention relates to *Brassica* sequences comprising seed- and funiculus-preferential promoter activity. Provided are recombinant genes comprising the seed- and funiculus-preferential promoter operably linked to a heterologous nucleic acid sequence, and cells, plants and seeds comprising the recombinant gene. The promoters can be used to alter gene expression preferentially in the seeds and in the funiculus and to alter biotic or abiotic stress tolerance, yield, seed quality or seed properties.

SEED- AND FUNICULUS-PREFERENTIAL PROMOTERS AND USES THEREOF

FIELD OF THE INVENTION

[1] The present invention relates to materials and methods for the expression of a gene of interest preferentially in seeds and in the funiculus of plants. In particular, the invention provides an expression cassette for
5 regulating seed- and funiculus-preferential expression in plants.

BACKGROUND

[2] Modification of plants to alter and/or improve phenotypic characteristics (such as productivity or quality) requires the overexpression or down-regulation of endogenous genes or the expression of heterologous genes in plant tissues. Such genetic modification relies on the availability of a means to drive and to control gene expression
10 as required. Indeed, genetic modification relies on the availability and use of suitable promoters which are effective in plants and which regulate gene expression so as to give the desired effect(s) in the transgenic plant.

[3] For numerous applications in plant biotechnology a tissue-specific or a tissue-preferential expression profile is advantageous, since beneficial effects of expression in one tissue may have disadvantages in others.

[4] Seed-preferential or seed-specific promoters are useful for expressing or down-regulating genes
15 specifically in the seeds to get the desired function or effect, such as improving disease resistance, herbicide resistance, modifying seed or grain composition or quality, such as modifying starch quality or quantity, modifying oil quality or quantity, modifying amino-acid or protein composition, improving tolerance to biotic or abiotic stress, increasing yield, or altering metabolic pathways in the seeds.

[5] Examples of seed-preferential or seed-specific promoters include the Tonoplast Intrinsic Protein alpha promoter from *Arabidopsis thaliana* (US patent application US2009/0241230), the KNAT411 promoter from *Arabidopsis thaliana* (US Pat. No. 6,342,657), an oleosin promoter, a 2S storage protein promoter or a legumin-like seed storage protein promoter from *Linum usitatissimum* (US Pat. No. 7,642,346), the acyl carrier protein promoter from *Brassica napus* (US Pat. Application No. 1994/0129129), the β -amylase promoter of barley (US Pat. Application No. 1997/0793599), and the Ha ds10 G1 promoter of sunflower (US Pat. No. 6,759,570).

[6] There remains thus an interest in the isolation of novel seed- and funiculus-preferential promoters having moderate promoter activity. It is thus an objective of the present invention to provide *Brassica* promoters having moderate seed- and funiculus-preferential activity. This objective is solved by the present invention as herein further explained.

SUMMARY

[7] In one aspect, the invention provides an isolated nucleic acid comprising seed- and funiculus-preferential promoter activity selected from the group consisting of (a) a nucleic acid comprising the nucleotide sequence selected from the group: (i) the nucleotide sequence from position 1 to position 1414 of SEQ ID NO: 3, or functional
5 fragment thereof, (ii) the nucleotide sequence from position 1 to position 1364 of SEQ ID NO: 4, or functional fragment thereof, (iii) the nucleotide sequence from position 1 to position 1365 of SEQ ID NO: 5, or functional fragment thereof, (iv) the nucleotide sequence from position 1 to position 1358 of SEQ ID NO: 6, or functional fragment thereof, and (v) the nucleotide sequence from position 1 to position 1363 of SEQ ID NO: 7, or functional
10 fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of (a), or a functional fragment thereof.

[8] In a further embodiment, the nucleic acid described above comprises the nucleotide sequence of SEQ ID NO: 18; the nucleotide sequence of SEQ ID NO: 19; the nucleotide sequence of SEQ ID NO: 20; and the nucleotide sequence of SEQ ID NO: 21.

[9] In another embodiment, said nucleic acid further comprises an intron, the nucleotide sequence of which is
15 selected from the group of: (a) a nucleotide sequence selected from the group: (i) the nucleotide sequence from position 1418 to position 2055 of SEQ ID NO: 3, or a functional fragment thereof, (ii) the nucleotide sequence from position 1368 to position 1650 of SEQ ID NO: 4, or a functional fragment thereof, (iii) the nucleotide sequence from position 1369 to position 2001 of SEQ ID NO: 5, or a functional fragment thereof, (iv) the nucleotide sequence from position 1362 to position 1647 of SEQ ID NO: 6, or a functional fragment thereof, and (v) the nucleotide sequence
20 from position 1367 to position 2001 of SEQ ID NO: 7, or a functional fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of (a), or a functional fragment thereof. The nucleic acid sequence of said intron comprises the nucleotide sequence of SEQ ID NO: 22, the nucleotide sequence of SEQ ID NO: 23, and the nucleotide sequence of SEQ ID NO: 24. In a further embodiment, said nucleic acid comprising the intron has higher seed- and funiculus- preferential promoter activity than the
25 nucleic acid not comprising the intron.

[10] In yet another embodiment the above described nucleic acid is selected from the group: (a) a nucleic acid comprising a nucleotide sequence of any one of SEQ ID NOs: 3 to 7 or a functional fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of SEQ ID NOs: 3 to 7 or a functional fragment thereof.

30

[11] A further embodiment provides a recombinant gene comprising the nucleic acid according to the invention operably linked to a heterologous nucleic acid sequence encoding an expression product of interest, and optionally a transcription termination and polyadenylation sequence, preferably a transcription termination and polyadenylation region functional in plant cells. In a further embodiment, said expression product of interest is an RNA capable of modulating the expression of a gene or is a protein.

[12] Yet another embodiment provides a host cell, such as an *E. coli* cell, an *Agrobacterium* cell, a yeast cell, an algal cell, or a plant cell, comprising the isolated nucleic acid according to the invention, or the recombinant gene according to the invention.

[13] In further embodiments, a plant and a plant cell are provided comprising the recombinant gene according to the invention. Yet a further embodiment provides seeds obtainable from the plant according to the invention. In another embodiment, the plants or plant parts according to the invention are seed crop plants or seeds.

[14] Yet another embodiment provides a method of producing a transgenic plant comprising the steps of (a) introducing or providing the recombinant gene according to the invention to a plant cell to create transgenic cells; and (b) regenerating transgenic plants from said transgenic cell.

[15] Further provided is a method of effecting seed- and funiculus-preferential expression of a nucleic acid comprising introducing the recombinant gene according to the invention into the genome of a plant, or providing the plant according to the invention. Also provided is a method for altering seed properties of a plant or to produce a commercially relevant product in a plant, said method comprising introducing the recombinant gene according to the invention into the genome of a plant, or providing the plant according to the invention. In another embodiment, said plant is a seed crop plant.

[16] Also provided is the use of the isolated nucleic acid according to the invention to regulate expression of an operably linked nucleic acid in a plant, and the use of the isolated nucleic acid according to the invention, or the recombinant gene according to the invention to alter seed properties of a plant or to produce a commercially relevant product in a plant. In a further embodiment, said plant is a seed crop plant.

[17] Yet another embodiment provides a method of producing food, feed, or an industrial product comprising (a) obtaining the plant or a part thereof, according to the invention; and (b) preparing the food, feed or industrial product from the plant or part thereof. In another embodiment, 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[18] Figure 1: Graphical display of the gene prediction for SLP1 BnA, using the FGeneSH prediction tool.

[19] Figure 2: Semi quantitative assessment of GUS labelling ($\mu\text{U}\cdot\text{mg}^{-1}$ fresh weight $\cdot\text{h}$) in transgenic lines

carrying a Psp1BnA::GUS T-DNA (A and B) or a Psp1BnAintron::GUS T-DNA (C and D). Embryos (A and C) and seed coats (B and D) were stained at the following stages: 1: 15 to 18 DAF, 2: 20 to 23 DAF, 3: 27 to 32 DAF, 4: 35 to 40 DAF. For the stages 1, 2 and 4 embryos, the stainings are assessed for the whole embryos while for the stage 3 embryos, the staining was quantified in the outer cotyledon (a), in the inner cotyledon (b) and in the hypocotyl (c) separately.

[20] Figure 3: Expression profile analysis in seeds carrying the T-DNA Psp1 intron BnA::GUS. GUS labelling in the seed coat and endosperm at early stage (A, B) and at late stage (C), as well as in the funiculus (D). The right panel of D represent a close-up view of one of the funiculi shown in the left panel of D.

[21] Figure 4: Alignment of the amino acid sequence of different Brassica SLP1 proteins. Amino acid residues conserved in all proteins are indicated by an asterisk, conserved amino acid substitutions are indicated by a colon. The lowest sequence identity between any two SPL1 protein is about 97%.

[22] Figure 5: Relative expression levels of different Brassica SLP1 transcripts in different plant tissues. A: SLP1 BnA; B: SLP1BnC; C: SPL1 Br; D: SLP1 Bo; E: SLP1 BjA. Different tissues for A to D: AM33: Apical meristem 33 days after sowing (DAS); BFB42: Big flower buds 42 DAS; CTYL10: Cotyledons 10 DAS; OF52: Open flowers 52 DAS; Pod2: Pods 14-20 DAS; Pod3: Pods 21-25 DAS; Ro2w: Roots 14 DAS; SFB42: Small flower buds 42 DAS; Seed2: Seeds 14-20 days after flowering (DAF); Seed3: Seeds 21-25 DAF; Seed4: Seeds 26-30 DAF; Seed5: Seeds 31-35 DAF; Seed6: Seeds 42 DAF; Seed7: Seeds 49 DAF; St2w: Stem 14 DAS; St5w: Stem 33 DAS; YL33: Young leaf 33 DAS. Different tissues for E: AM22: Apical meristem 22 days after sowing (DAS); BFB35: Big flower buds 35 DAS; CTYL8: Cotyledons 8 DAS; OF35: Open flowers 35 DAS; Pod2: Pods 14-20 DAS; Pod3: Pods 21-25 DAS; Pod4: Pods 26-30 DAS; Pod5: Pods 31-35 DAS; Ro2w: Roots 14 DAS; SFB35: Small flower buds 35 DAS; Seed2: Seeds 14-20 days after flowering (DAF); Seed3: Seeds 21-25 DAF; Seed4: Seeds 26-30 DAF; Seed5: Seeds 31-35 DAF; Seed6: Seeds 42 DAF; Seed7: Seeds 49 DAF; St2w: Stem 14 DAS; St3w: Stem 22 DAS; YL22: Young leaf 22 DAS; OL22: Old leaf 22 DAS.

[23] Figure 6: Alignment of the about 400 nucleotides upstream of the translation start of the nucleotide sequence of the Brassica Psp1 promoters from *Brassica napus*, *Brassica juncea*, *Brassica oleracea* and *Brassica rapa*. The TATA Box is indicated by a frame. The transcription initiation start is indicated in bold. The consensus sequences are underlined and named. Promoter consensus sequence has the sequence of SEQ ID NO: 18, UTR consensus sequence 1 has the sequence of SEQ ID NO: 19, UTR consensus sequence 2 has the sequence of SEQ ID NO: 20, UTR consensus sequence 3 has the sequence of SEQ ID NO: 21.

[24] Figure 7: Alignment of the translation start codons and of the introns of the nucleotide sequence of the Brassica Psp1 promoters from *Brassica napus*, *Brassica juncea*, *Brassica oleracea* and *Brassica rapa*. The translation start codon is indicated in bold. The consensus sequences are underlined and named. Intron consensus sequence 1 has the sequence of SEQ ID NO: 22, intron consensus sequence 2 has the sequence of SEQ ID NO: 23, intron consensus sequence 3 has the sequence of SEQ ID NO: 24.

DETAILED DESCRIPTION

[25] The present invention is based on the observation that SEQ ID NOs: 3 to 7 have seed- and funiculus-preferential promoter activity in *Brassica*.

[26] SEQ ID NOs: 3 to 7 depicts the region upstream (i.e. located 5' upstream of) from the first ATG start codon plus the first intron following the ATG of the SLP1 BnA, SLP1 BnC, SLP1 Br, SLP1 Bo, SLP1 BJA genes respectively.

[27] SLP1 BnA and SLP1 BnC are the two copies present in *Brassica napus* of the orthologous gene to the *Arabidopsis thaliana* SLP1 gene At1g60970, which encodes a SNARE-like superfamily protein. SLP1 Br represents the two identical copies present in *Brassica rapa* of the orthologous gene to the *Arabidopsis thaliana* SLP1 gene At1g60970. SLP1 Bo is the only copy present in *Brassica oleracea* of the orthologous gene to the *Arabidopsis thaliana* SLP1 gene At1g60970. SLP1 BJA is one of the two copies present in *Brassica juncea* of the orthologous gene to the *Arabidopsis thaliana* SLP1 gene At1g60970. The SNARE (N-ethylmaleimide-sensitive factor adaptor protein receptor) gene family is a large family of genes encoding membrane associated proteins identified as key players in vesicle trafficking and vesicle fusion. Different members of this family are involved in diverse biological processes in plants, like for example cytokinesis, stress response and shoot gravitropism. Little is known about the expression pattern of the different members of this super family; however, *in silico* expression analysis in *Arabidopsis* indicated that the different members have different profiles, with most being either ubiquitously expressed, or preferentially expressed in the pollen (reviewed in Lipka et al. 2007).

[28] In one aspect, the invention provides an isolated nucleic acid comprising seed- and funiculus-preferential promoter activity selected from the group consisting of (a) a nucleic acid comprising the nucleotide sequence selected from the group: (i) the nucleotide sequence from position 1 to position 1414 of SEQ ID NO: 3, or functional fragment thereof, (ii) the nucleotide sequence from position 1 to position 1364 of SEQ ID NO: 4, or functional fragment thereof, (iii) the nucleotide sequence from position 1 to position 1365 of SEQ ID NO: 5, or functional fragment thereof, (iv) the nucleotide sequence from position 1 to position 1358 of SEQ ID NO: 6, or functional fragment thereof, and (v) the nucleotide sequence from position 1 to position 1363 of SEQ ID NO: 7, or functional fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of (a), or a functional fragment thereof.

[29] In a further embodiment, the nucleic acid described above comprises the nucleotide sequence of SEQ ID NO: 18; the nucleotide sequence of SEQ ID NO: 19; the nucleotide sequence of SEQ ID NO: 20; and the nucleotide sequence of SEQ ID NO: 21.

[30] In another embodiment, said nucleic acid further comprises an intron, the nucleotide sequence of which is selected from the group of: (a) a nucleotide sequence selected from the group: (i) the nucleotide sequence from position 1418 to position 2055 of SEQ ID NO: 3, or a functional fragment thereof, (ii) the nucleotide sequence from

position 1368 to position 1650 of SEQ ID NO: 4, or a functional fragment thereof, (iii) the nucleotide sequence from position 1369 to position 2001 of SEQ ID NO: 5, or a functional fragment thereof, (iv) the nucleotide sequence from position 1362 to position 1647 of SEQ ID NO: 6, or a functional fragment thereof, and (v) the nucleotide sequence from position 1367 to position 2001 of SEQ ID NO: 7, or a functional fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of (a), or a functional fragment thereof. The nucleic acid sequence of said intron comprises the nucleotide sequence of SEQ ID NO: 22, the nucleotide sequence of SEQ ID NO: 23, and the nucleotide sequence of SEQ ID NO: 24. In a further embodiment, said nucleic acid comprising the intron has higher seed- and funiculus- preferential promoter activity than the nucleic acid not comprising the intron.

10 [31] In yet another embodiment the above described nucleic acid is selected from the group: (a) a nucleic acid comprising a nucleotide sequence of any one of SEQ ID NOs: 3 to 7 or a functional fragment thereof; and (b) a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of SEQ ID NOs: 3 to 7 or a functional fragment thereof.

[32] The nucleic acid comprising the seed- and funiculus-preferential promoter activity according to the invention may also be comprised in a larger DNA molecule.

[33] "Seed- and funiculus-preferential promoter activity" in the context of this invention means the promoter activity is at least 2 times, or at least 5 times, or at least 10 times, or at least 20 times, or at least 50 times, or even at least 100 times higher in seeds and in the funiculus than in other tissues. In other words, in seed- and funiculus-preferential promoter activity, transcription of the nucleic acid operably linked to the promoter of the invention in the seeds and the funiculus is at least 2 times, or at least 5 times, or at least 10 times, or at least 20 times, or at least 50 times or even at least 100 times higher than in other tissues. In other words, the seed- and funiculus-preferential promoter drives seed- and funiculus-preferential expression of the nucleic acid operably linked to the seed- and funiculus-preferential promoter.

[34] The phrase "operably linked" refers to the functional spatial arrangement of two or more nucleic acid regions or nucleic acid sequences. For example, a promoter region may be positioned relative to a nucleic acid sequence such that transcription of a nucleic acid sequence is directed by the promoter region. Thus, a promoter region is "operably linked" to the nucleic acid sequence. "Functionally linked" is an equivalent term.

[35] The phrases "DNA", "DNA sequence," "nucleic acid sequence," "nucleic acid molecule" "nucleotide sequence" and "nucleic acid" refer to a physical structure comprising an orderly arrangement of nucleotides. The DNA sequence or nucleotide sequence may be contained within a larger nucleotide molecule, vector, or the like. In addition, the orderly arrangement of nucleic acids in these sequences may be depicted in the form of a sequence listing, figure, table, electronic medium, or the like.

[36] As used herein, "promoter" means a region of DNA sequence that is essential for the initiation of transcription of DNA, resulting in the generation of an RNA molecule that is complementary to the transcribed DNA;

this region may also be referred to as a "5' regulatory region." Promoters are usually located upstream of the coding sequence to be transcribed and have regions that act as binding sites for RNA polymerase II and other proteins such as transcription factors (trans-acting protein factors that regulate transcription) to initiate transcription of an operably linked gene. Promoters may themselves contain sub-elements (i.e. promoter motifs) such as cis-elements or enhancer domains that regulate the transcription of operably linked genes. The promoters of this invention may be altered to contain "enhancer DNA" to assist in elevating gene expression. As is known in the art, certain DNA elements can be used to enhance the transcription of DNA. These enhancers often are found 5' to the start of transcription in a promoter that functions in eukaryotic cells, but can often be inserted upstream (5') or downstream (3') to the coding sequence. In some instances, these 5' enhancer DNA elements are introns. Among the introns that are useful as enhancer DNA are the 5' introns from the rice actin 1 gene (see US5641876), the rice actin 2 gene, the maize alcohol dehydrogenase gene, the maize heat shock protein 70 gene (see US5593874), the maize shrunken 1 gene, the light sensitive 1 gene of *Solanum tuberosum*, the Arabidopsis histon 4 intron and the heat shock protein 70 gene of *Petunia hybrida* (see US5659122). Thus, as contemplated herein, a promoter or promoter region includes variations of promoters derived by inserting or deleting regulatory regions, subjecting the promoter to random or site-directed mutagenesis, etc. The activity or strength of a promoter may be measured in terms of the amounts of RNA it produces, or the amount of protein accumulation in a cell or tissue, relative to a promoter whose transcriptional activity has been previously assessed or relative to a promoter driving the expression of a housekeeping gene.

[37] A promoter as used herein may thus include sequences downstream of the transcription start, such as sequences coding the 5' untranslated region (5' UTR) of the RNA, introns located downstream of the transcription start, or even sequences encoding the protein. A functional promoter fragment according to the invention may comprise its own 5'UTR comprising the nucleotide sequence of SEQ ID NO: 3 from nucleotide 1187 to nucleotide 1414, or comprising the nucleotide sequence of SEQ ID No: 4 from nucleotide 1143 to nucleotide 1364, or comprising the nucleotide sequence of SEQ ID No: 5 from nucleotide 1138 to nucleotide 1365, or comprising the nucleotide sequence of SEQ ID No: 6 from nucleotide 1134 to nucleotide 1358, or comprising the nucleotide sequence of SEQ ID No: 7 from nucleotide 1124 to nucleotide 1363. Alternatively, 5'UTR fragments from other Brassica SLP1 genes may be used. For example, a promoter fragment of SEQ ID NO: 3 may have the nucleotide sequence of said sequence from position 1187 to 1414 replaced by the nucleotide sequence of SEQ ID NO: 4 from nucleotide 1143 to nucleotide 1364. A promoter fragment of SEQ ID NO: 3 may have the nucleotide sequence of said sequence from position 1187 to 1414 replaced by the nucleotide sequence of SEQ ID NO: 5 from nucleotide 1138 to nucleotide 1365. A promoter fragment of SEQ ID NO: 3 may have the nucleotide sequence of said sequence from position 1187 to 1414 replaced by the nucleotide sequence of SEQ ID NO: 6 from nucleotide 1134 to nucleotide 1358. A promoter fragment of SEQ ID NO: 3 may have the nucleotide sequence of said sequence from position 1187 to 1414 replaced by the nucleotide sequence of SEQ ID NO: 7 from nucleotide 1124 to nucleotide 1363. As another example, a promoter fragment of SEQ ID NO: 4 may have the nucleotide sequence of said sequence from position 1143 to position 1364 replaced by the nucleotide sequence of SEQ ID NO: 3 from nucleotide 1187 to nucleotide 1414. A promoter fragment of SEQ ID NO: 4 may have the nucleotide sequence of

said sequence from position 1143 to position 1364 replaced by the nucleotide sequence of SEQ ID NO: 5 from nucleotide 1138 to nucleotide 1365. A promoter fragment of SEQ ID NO: 4 may have the nucleotide sequence of said sequence from position 1143 to position 1364 replaced by the nucleotide sequence of SEQ ID NO: 6 from nucleotide 1134 to nucleotide 1358. A promoter fragment of SEQ ID NO: 4 may have the nucleotide sequence of said sequence from position 1143 to position 1364 replaced by the nucleotide sequence of SEQ ID NO: 7 from nucleotide 1124 to nucleotide 1363. As yet another example, a promoter fragment of SEQ ID NO: 5 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 3 from nucleotide 1187 to nucleotide 1414. A promoter fragment of SEQ ID NO: 5 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 4 from nucleotide 1143 to nucleotide 1364. A promoter fragment of SEQ ID NO: 5 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 6 from nucleotide 1134 to nucleotide 1358. A promoter fragment of SEQ ID NO: 5 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 7 from nucleotide 1124 to nucleotide 1363. As another example, a promoter fragment of SEQ ID NO: 6 may have the nucleotide sequence of said sequence from position 1134 to position 1358 replaced by the nucleotide sequence of SEQ ID NO: 3 from nucleotide 1187 to nucleotide 1414. A promoter fragment of SEQ ID NO: 6 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 4 from nucleotide 1143 to nucleotide 1364. A promoter fragment of SEQ ID NO: 6 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 5 from nucleotide 1138 to nucleotide 1365. A promoter fragment of SEQ ID NO: 6 may have the nucleotide sequence of said sequence from position 1138 to position 1365 replaced by the nucleotide sequence of SEQ ID NO: 7 from nucleotide 1124 to nucleotide 1363. As another example, a promoter fragment of SEQ ID NO: 7 may have the nucleotide sequence of said sequence from position 1124 to position 1363 replaced by the nucleotide sequence of SEQ ID NO: 4 from nucleotide 1143 to nucleotide 1364. A promoter fragment of SEQ ID NO: 7 may have the nucleotide sequence of said sequence from position 1124 to position 1363 replaced by the nucleotide sequence of SEQ ID NO: 5 from nucleotide 1138 to nucleotide 1365. A promoter fragment of SEQ ID NO: 7 may have the nucleotide sequence of said sequence from position 1124 to position 1363 replaced by the nucleotide sequence of SEQ ID NO: 6 from nucleotide 1134 to nucleotide 1358.

[38] A promoter fragment according to the invention may comprise its own intron comprising the nucleotide sequence of SEQ ID NO: 3 from nucleotide 1418 to nucleotide 2055, or comprising the nucleotide sequence of SEQ ID No: 4 from nucleotide 1368 to nucleotide 1650, or comprising the nucleotide sequence of SEQ ID No: 5 from nucleotide 1369 to nucleotide 2001, or comprising the nucleotide sequence of SEQ ID No: 6 from nucleotide 1362 to nucleotide 1647, or comprising the nucleotide sequence of SEQ ID No: 7 from nucleotide 1367 to nucleotide 2001. Alternatively, intron fragments from other Brassica SLP1 genes may be used. For example, a promoter fragment of SEQ ID NO: 3 may have the nucleotide sequence of said sequence from position 1418 to

have the nucleotide sequence of said sequence from position 1367 to position 2001 replaced by the nucleotide sequence of SEQ ID NO: 6 from nucleotide 1362 to nucleotide 1647.

[39] Such a promoter fragment may be at least about 400 bp, at least about 500 bp, at least about 600 bp, at least about 700 bp, at least about 800 bp, at least about 900 bp, at least about 1000 bp, at least about 1100 bp, at least about 1200 bp, or at least about 1300 bp upstream of the first ATG start codon of the SLP1 transcripts and have seed- and funiculus-preferential promoter activity. In combination with the above described promoter fragments, a promoter fragment according to the invention may thus comprise the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 1014 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 914 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 814 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 714 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 614 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 514 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 414 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 314 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 214 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 114 to the nucleotide at position 1414, the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 14 to the nucleotide at position 1414, or the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 1 to the nucleotide at position 1414. A promoter fragment according to the invention may also comprise the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 964 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 864 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 764 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 664 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 564 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 464 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 364 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 264 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 164 to the nucleotide at position 1364, the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 64 to the nucleotide at position 1364, or the nucleotide sequence of SEQ ID No: 4 from the nucleotide at position 1 to the nucleotide at position 1364. A promoter fragment according to the invention may also comprise the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 965 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 865 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 765 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 665 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 565 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 465 to the nucleotide at position 1365, the nucleotide

sequence of SEQ ID No: 5 from the nucleotide at position 365 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 265 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 165 to the nucleotide at position 1365, the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 65 to the nucleotide at position 1365, or the nucleotide sequence of SEQ ID No: 5 from the nucleotide at position 1 to the nucleotide at position 1365. A promoter fragment according to the invention may also comprise the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 958 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 858 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 758 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 658 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 558 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 458 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 358 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 258 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 158 to the nucleotide at position 1358, the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 58 to the nucleotide at position 1358, or the nucleotide sequence of SEQ ID No: 6 from the nucleotide at position 1 to the nucleotide at position 1358. A promoter fragment according to the invention may also comprise the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 963 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 863 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 763 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 663 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 563 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 463 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 363 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 263 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 163 to the nucleotide at position 1363, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 63 to the nucleotide at position 1363, or the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 1 to the nucleotide at position 1363.

[40] Such a promoter fragment may be at least about 400 bp, at least about 500 bp, at least about 600 bp, at least about 700 bp, at least about 800 bp, at least about 900 bp, at least about 1000 bp, at least about 1100 bp, at least about 1200 bp, or at least about 1300 bp upstream of the first ATG start codon of the SLP1 transcripts and at least about 300 bp, at least about 400 bp, at least about 500 bp or at least about 600 bp of the intron downstream of the first ATG start codon of the SLP1 gene and have seed- and funiculus-preferential promoter activity. In such promoter fragments the first ATG start codon may optionally be present immediately before the intron fragment. In combination with the above described promoter fragments, a promoter fragment according to the invention may thus comprise the nucleotide sequence of SEQ ID NO: 3 from the nucleotide at position 1014 to the nucleotide at position 1414 and from the nucleotide position 1418 to the nucleotide position 1718, the nucleotide sequence of

from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 763 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 663 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 563 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 463 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 363 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 263 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 163 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 63 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001, or the nucleotide sequence of SEQ ID No: 7 from the nucleotide at position 1 to the nucleotide at position 1363 and from the nucleotide at position 1367 to the nucleotide at position 2001.

[41] Promoter activity for a functional promoter fragment in seeds may be determined by those skilled in the art, for example using analysis of RNA accumulation produced from the nucleic acid which is operably linked to the promoter as described herein, whereby the nucleic acid which is operably linked to the promoter can be the nucleic acid which is naturally linked to the promoter, i.e. the endogenous gene of which expression is driven by the promoter.

[42] The seed- and funiculus-preferential expression capacity of the identified or generated fragments of the promoters of the invention can be conveniently tested by determining levels of the transcript of which expression is naturally driven by the promoter of the invention, i.e. endogenous transcript levels, such as, for example, using the methods as described herein in the Examples. Further, the seed- and funiculus-preferential expression capacity of the identified or generated fragments of the promoters of the invention can be conveniently tested by operably linking such DNA molecules to a nucleotide sequence encoding an easy scorable marker, e.g. a beta-glucuronidase gene, introducing such a chimeric gene into a plant and analyzing the expression pattern of the marker in seeds and in the funiculus as compared with the expression pattern of the marker in other parts of the plant. Other candidates for a marker (or a reporter gene) are chloramphenicol acetyl transferase (CAT) and proteins with fluorescent properties, such as green fluorescent protein (GFP) from *Aequora victoria* or proteins with luminescent properties such as the *Renilla* luciferase or the bacterial *lux* operon. To define a minimal promoter region, a DNA segment representing the promoter region is removed from the 5' region of the gene of interest and operably linked to the coding sequence of a marker (reporter) gene by recombinant DNA techniques well known to the art. The reporter gene is operably linked downstream of the promoter, so that transcripts initiating at the promoter proceed through the reporter gene. Reporter genes generally encode proteins, which are easily measured, including, but not

- 21 -

limited to, chloramphenicol acetyl transferase (CAT), beta-glucuronidase (GUS), green fluorescent protein (GFP), beta-galactosidase (beta-GAL), and luciferase. The expression cassette containing the reporter gene under the control of the promoter can be introduced into an appropriate cell type by transfection techniques well known to the art. To assay for the reporter protein, cell lysates are prepared and appropriate assays, which are well known in the art, for the reporter protein are performed. For example, if CAT were the reporter gene of choice, the lysates from cells transfected with constructs containing CAT under the control of a promoter under study are mixed with isotopically labeled chloramphenicol and acetyl-coenzyme A (acetyl-CoA). The CAT enzyme transfers the acetyl group from acetyl-CoA to the 2- or 3-position of chloramphenicol. The reaction is monitored by thin-layer chromatography, which separates acetylated chloramphenicol from unreacted material. The reaction products are then visualized by autoradiography. The level of enzyme activity corresponds to the amount of enzyme that was made, which in turn reveals the level of expression and the seed-preferential functionality from the promoter or promoter fragment of interest. This level of expression can also be compared to other promoters to determine the relative strength of the promoter under study. Once activity and functionality is confirmed, additional mutational and/or deletion analyses may be employed to determine the minimal region and/or sequences required to initiate transcription. Thus, sequences can be deleted at the 5' end of the promoter region and/or at the 3' end of the promoter region, and nucleotide substitutions introduced. These constructs are then again introduced in cells and their activity and/or functionality determined.

[43] The activity or strength of a promoter may be measured in terms of the amount of mRNA or protein accumulation it specifically produces, relative to the total amount of mRNA or protein. The promoter preferably expresses an operably linked nucleic acid sequence at a level greater than about 0.001%, about 0.002%, more preferably greater than about 0.005% of the total mRNA. Alternatively, the activity or strength of a promoter may be expressed relative to a well-characterized promoter (for which transcriptional activity was previously assessed).

[44] It will herein further be clear that equivalent seed- and funiculus-preferential promoters can be isolated from other plants. To this end, equivalent promoters can be isolated using the coding sequences of the genes driven by the promoters of any one of SEQ ID NOs: 3 to 7 to screen a genomic library (e.g. by hybridization or *in silico*) of a crop of interest. When sufficient identity between the coding sequences is obtained (for example, higher than 85% identity) then promoter regions can be isolated upstream of the orthologous genes.

[45] Suitable to the invention are nucleic acids comprising seed- and funiculus-preferential promoter activity which comprise a nucleotide sequence having at least 40%, at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 98% sequence identity to the herein described promoters and promoter regions or functional fragments thereof and are also referred to as variants. The term "variant" with respect to the transcription regulating nucleotide sequences SEQ ID NOs: 3 to 7 of the invention is intended to mean substantially similar sequences. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as herein outlined before. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed

mutagenesis of any one of SEQ ID NOs: 3 to 7. Generally, nucleotide sequence variants of the invention will have at least 40%, 50%, 60%, to 70%, e.g., preferably 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, to 79%, generally at least 80%, e.g., 81% to 84%, at least 85%, e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, to 98% and 99% nucleotide sequence identity to the native (wild type or endogenous) nucleotide sequence or a functional fragment thereof. Derivatives of the DNA molecules disclosed herein may include, but are not limited to, deletions of sequence, single or multiple point mutations, alterations at a particular restriction enzyme site, addition of functional elements, or other means of molecular modification which may enhance, or otherwise alter promoter expression. Techniques for obtaining such derivatives are well-known in the art (see, for example, J. F. Sambrook, D. W. Russell, and N. Irwin (2000) *Molecular Cloning: A Laboratory Manual*, 3rd edition Volumes 1, 2, and 3. Cold Spring Harbor Laboratory Press). For example, one of ordinary skill in the art may delimit the functional elements within the promoters disclosed herein and delete any non-essential elements. Functional elements may be modified or combined to increase the utility or expression of the sequences of the invention for any particular application. Those of skill in the art are familiar with the standard resource materials that describe specific conditions and procedures for the construction, manipulation, and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), as well as the generation of recombinant organisms and the screening and isolation of DNA molecules. As used herein, the term "percent sequence identity" refers to the percentage of identical nucleotides between two segments of a window of optimally aligned DNA. Optimal alignment of sequences for aligning a comparison window are well-known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman (Waterman, M. S. *Introduction to Computational Biology: Maps, sequences and genomes*. Chapman & Hall. London (1995), the homology alignment algorithm of Needleman and Wunsch (*J. Mol. Biol.*, 48:443-453 (1970), the search for similarity method of Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444 (1988), and preferably by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG (Registered Trade Mark), Wisconsin Package (Registered Trade Mark from Accelrys Inc., San Diego, Calif.). An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components that are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, i.e., the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction times 100. The comparison of one or more DNA sequences may be to a full-length DNA sequence or a portion thereof, or to a longer DNA sequence.

[46] A nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of SEQ ID NO: 3 to 7 can thus be a nucleic acid comprising a nucleotide sequence having at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 98%, or 100% sequence identity to any one of SEQ ID NOs: 3 to 7.

[47] A "functional fragment" of a nucleic acid comprising seed-preferential promoter denotes a nucleic acid comprising a stretch of the nucleic acid sequences of any one of SEQ ID NOs: 3 to 7, or of the nucleic acid having at least 80% sequence identity to any one of SEQ ID NOs: 3 to 7 which still exerts the desired function, i.e. which has seed-preferential promoter activity. Assays for determining seed-preferential promoter activity are provided

herein. Preferably, the functional fragment of the seed-preferential promoter contains the conserved promoter motifs, such as, for example, conserved promoter motifs as described in DoOP (doop.abc.hu, databases of Orthologous Promoters, Barta E. *et al* (2005) *Nucleic Acids Research* Vol. 33, D86-D90). A functional fragment may be a fragment of at least about 400 bp, at least about 500 bp, at least about 600 bp, at least about 700 bp, at least about 800 bp, at least about 900 bp, at least about 1000 bp, at least about 1100 bp; at least about 1200 bp, or at least about 1300 bp upstream of the first ATG start codon of the SLP1 transcripts and at least about 300 bp, at least about 400 bp, at least about 500 bp or at least about 600 bp downstream of the first ATG start codon of the SLP1 gene and have seed- and funiculus-preferential promoter activity.

[48] A nucleic acid comprising the nucleotide sequence of any one of SEQ ID NO: 3 to 7 which further comprises insertion, deletion, substitution of at least 1 nucleotide up to 20 nucleotides, at least 1 nucleotide up to 15 nucleotides, at least 1 nucleotide up to 10 nucleotides, at least 1 nucleotide up to 5 nucleotides, at least 1 nucleotide up to 4 nucleotides, at least 1 nucleotide up to 3 nucleotides, or even at least 1 nucleotide up to 2 nucleotides may cover at least about 300 bp, at least about 400 bp, at least about 500 bp, at least about 600 bp, at least about 700 bp, at least about 800 bp, at least about 900 bp, at least about 1000 bp, at least about 1100 bp; at least about 1200 bp, or at least about 1300 bp from the translation start site.

[49] A number of highly conserved regions (consensus sequences) were identified on the promoter sequence disclosed herein.

[50] Variants of the promoter described herein include those which comprise the identified consensus sequences – promoter consensus sequence (SEQ ID NO: 18), UTR consensus sequence 1 (SEQ ID NO: 19), UTR consensus sequence 2 (SEQ ID NO: 20), UTR consensus sequence 3 (SEQ ID NO: 21), intron consensus sequence 1 (SEQ ID NO: 22), intron consensus sequence 2 (SEQ ID NO: 23) and/or intron consensus sequence 3 (SEQ ID NO: 24)-, but have otherwise been modified to delete nucleotide stretches within the sequence which are not needed for the promoter to be functional in seed- and funiculus-preferential manner. For example, any nucleotide stretch located between the consensus sequences and /or between the transcriptional start and the first consensus sequence may be at least partially deleted to result in a shorter nucleotide sequence than the about 2 kb sequence of SEQ ID NOs: 3, 5 and 7, or than the about 1.7 kb of SEQ ID NOs: 4 and 6.

[51] "Isolated nucleic acid", used interchangeably with "isolated DNA" as used herein refers to a nucleic acid not occurring in its natural genomic context, irrespective of its length and sequence. Isolated DNA can, for example, refer to DNA which is physically separated from the genomic context, such as a fragment of genomic DNA. Isolated DNA can also be an artificially produced DNA, such as a chemically synthesized DNA, or such as DNA produced via amplification reactions, such as polymerase chain reaction (PCR) well-known in the art. Isolated DNA can further refer to DNA present in a context of DNA in which it does not occur naturally. For example, isolated DNA can refer to a piece of DNA present in a plasmid. Further, the isolated DNA can refer to a piece of DNA present in another chromosomal context than the context in which it occurs naturally, such as for example at another position in the genome than the natural position, in the genome of another species than the species in which it occurs

naturally, or in an artificial chromosome.

[52] A further embodiment provides a recombinant gene comprising the nucleic acid according to the invention operably linked to a heterologous nucleic acid sequence encoding an expression product of interest, and optionally a transcription termination and polyadenylation sequence, preferably a transcription termination and polyadenylation region functional in plant cells. In a further embodiment, said expression product of interest an RNA capable of modulating the expression of a gene or is a protein.

[53] The term "expression product" refers to a product of transcription. Said expression product can be the transcribed RNA. It is understood that the RNA which is produced is a biologically active RNA. Said expression product can also be a peptide, a polypeptide, or a protein, when said biologically active RNA is an mRNA and said protein is produced by translation of said mRNA.

[54] Alternatively, the heterologous nucleic acid, operably linked to the promoters of the invention, may also code for an RNA capable of modulating the expression of a gene. Said RNA capable of modulating the expression of a gene can be an RNA which reduces expression of a gene. Said RNA can reduce the expression of a gene for example through the mechanism of RNA-mediated gene silencing.

[55] Said RNA capable of modulating the expression of a gene can be a silencing RNA down-regulating expression of a target gene. As used herein, "silencing RNA" or "silencing RNA molecule" refers to any RNA molecule, which upon introduction into a plant cell, reduces the expression of a target gene. Such silencing RNA may e.g. be so-called "antisense RNA", whereby the RNA molecule comprises a sequence of at least 20 consecutive nucleotides having 95% sequence identity to the complement of the sequence of the target nucleic acid, preferably the coding sequence of the target gene. However, antisense RNA may also be directed to regulatory sequences of target genes, including the promoter sequences and transcription termination and polyadenylation signals. Silencing RNA further includes so-called "sense RNA" whereby the RNA molecule comprises a sequence of at least 20 consecutive nucleotides having 95% sequence identity to the sequence of the target nucleic acid. Other silencing RNA may be "unpolyadenylated RNA" comprising at least 20 consecutive nucleotides having 95% sequence identity to the complement of the sequence of the target nucleic acid, such as described in WO01/12824 or US6423885 (both documents herein incorporated by reference). Yet another type of silencing RNA is an RNA molecule as described in WO03/076619 (herein incorporated by reference) comprising at least 20 consecutive nucleotides having 95% sequence identity to the sequence of the target nucleic acid or the complement thereof, and further comprising a largely-double stranded region as described in WO03/076619 (including largely double stranded regions comprising a nuclear localization signal from a viroid of the Potato spindle tuber viroid-type or comprising CUG trinucleotide repeats). Silencing RNA may also be double stranded RNA comprising a sense and antisense strand as herein defined, wherein the sense and antisense strand are capable of base-pairing with each other to form a double stranded RNA region (preferably the said at least 20 consecutive nucleotides of the sense and antisense RNA are complementary to each other). The sense and antisense region may also be present within one RNA molecule such that a hairpin RNA (hpRNA) can be formed

when the sense and antisense region form a double stranded RNA region. hpRNA is well-known within the art (see e.g. WO99/53050, herein incorporated by reference). The hpRNA may be classified as long hpRNA, having long, sense and antisense regions which can be largely complementary, but need not be entirely complementary (typically larger than about 200 bp, ranging between 200 and 1000 bp). hpRNA can also be rather small ranging in size from about 30 to about 42 bp, but not much longer than 94 bp (see WO04/073390, herein incorporated by reference). Silencing RNA may also be artificial micro-RNA molecules as described e.g. in WO2005/052170, WO2005/047505 or US 2005/0144667, or ta-siRNAs as described in WO2006/074400 (all documents incorporated herein by reference). Said RNA capable of modulating the expression of a gene can also be an RNA ribozyme.

[56] Said RNA capable of modulating the expression of a gene can modulate, preferably down-regulate, the expression of other genes (i.e. target genes) comprised within the seeds or funiculus or even of genes present within a pathogen or pest that feeds upon the seeds of the transgenic plant such as a virus, fungus, insect, bacteria.

[57] The nucleic acid sequence heterologous to the promoters according to the invention may generally be any nucleic acid sequence effecting increased, altered (e.g. in a different organ) or reduced level of transcription of a gene for which such expression modulation is desired. The nucleic acid sequence can for example encode a protein of interest. Exemplary genes for which an increased or reduced level of transcription may be desired in the seeds are e.g. nucleic acids that can provide an agriculturally or industrially important feature in seeds. Suitable heterologous nucleic acid sequences of interest include nucleic acids modulating expression of genes conferring resistance to diseases, stress tolerance genes, genes involved in at different stages of fatty acid biosynthesis or degradation, in acyl editing, in storage compound storage or breakdown, genes encoding epoxidases, hydroxylases, cytochrome P450 mono-oxygenases, desaturases, tocopherol biosynthetic enzymes, carotenoid biosynthesis enzymes, amino acid biosynthetic enzymes, steroid pathway enzymes, starch branching enzymes, genes encoding proteins involved in starch synthesis, glycolysis, carbon metabolism, oxidative pentose phosphate cycle, protein synthesis, organelle organization and biogenesis, DNA metabolism, DNA replication, cell cycle, cell organization and biogenesis, cell proliferation, chromosome organization and biogenesis, microtubule-based processes, microtubule-based movement, cytoskeleton-dependent intracellular transport, cytoskeleton organization and biogenesis, chromatin assembly or disassembly, DNA-dependent DNA replication, chromosome organization and biogenesis, DNA packaging, establishment and/or maintenance of chromatin architecture, regulation of progression through the cell cycle, regulation of the cell cycle, nucleobase, nucleoside, nucleotide and nucleic acid metabolism, chromatin assembly, macromolecule biosynthesis, intracellular transport, establishment of cellular localization, cellular localization, nucleosome assembly, macromolecule metabolism, or M-phase; genes involved in secondary metabolism or genes involved in seed and/or seed coat architecture.

[58] Genes involved in the fatty acid biosynthesis or degradation include but are not limited to genes encoding an acyl-CoA synthetase, a glycerol-phosphate acyltransferase, an O-acyltransferase, a lyso-phosphatidic acid acyltransferase, a phosphatidic acid phosphatase, a diacylglycerol acyltransferase, an oleate desaturases, a linoleate desaturases, an acyl-CoA hydroxylase, an acyl-lipid hydroxylase, a fatty acid epoxidase, a phospholipid:sterol acyltransferase, a phospholipid:diacylglycerol acyltransferase, a diacylglycerol transacylase, a

lysophosphatidylcholine acyltransferase, a phosphatidylcholine:diacylglycerol cholinephosphotransferase, an acyl-CoA elongase, an acyl-lipid elongase, a phosphatidylglycerol-phosphate synthetase, a phosphatidylglycerol-phosphate phosphatase, a CDP-diacylglycerol synthetase, a phosphatidylinositol synthase, a phosphatidylserine synthase, a choline kinase, an ethanolamine kinase, a CDP-choline synthetase, a CDP-ethanolamine synthetase, a
 5 phosphatidylserine decarboxylase, a lipoxygenase, a phospholipase, a lipase, a carboxylesterase, a fatty alcohol reductase, a wax ester synthase, a bifunctional acyltransferases/wax synthase, a ketoacyl-CoA synthase, a ketoacyl-CoA reductase, a hydroxylacyl-CoA dehydrase, an enoyl-CoA reductase, an alcohol-forming fatty acyl-CoA reductase, an aldehyde-forming fatty acyl-CoA reductase, an aldehyde decarbonylase, a wax ester hydrolase, a glycerol-3-P-dehydrogenase, a CDP-choline:1,2-diacylglycerol cholinephosphotransferase, an oxidase, a
 10 ketosphinganine reductase, a ceramide synthase, an acylglycerophosphorylcholine acyltransferase, an acylglycerol-phosphate acyltransferase, a phosphoethanolamine N-methyltransferase, a ceramide sphingobase desaturase, a glucosylceramide synthase, a acyl-ceramide synthase, a triacylglycerol lipase, a monoacylglycerol lipase, an acyl-CoA oxidase, an hydroxyacyl-CoA dehydrogenase, a dienoyl-CoA reductase, a fatty acid omega-alcohol oxidase, a monoacylglycerol lipase, an acyl-CoA oxidase, a hydroxyacyl-CoA dehydrogenase, a dienoyl-
 15 CoA reductase, a fatty acid omega-alcohol oxidase, a fatty acid/acyl-CoA transporter, a acyl-CoA dehydrogenase, a diacylglycerol-phosphate kinase, a lysophosphatidic acid phosphatase, a peroxygenase; a $\Delta 4$ -desaturase; a $\Delta 5$ -desaturase, a $\Delta 6$ -desaturase; a $\Delta 9$ -desaturase, a $\Delta 12$ -desaturase or a $\Delta 15$ -desaturase.

[59] Genes involved in cell proliferation include but are not limited to genes encoding Da1 (Li et al., 2008, Genes Dev 22:1331, WO2015/067943), Da2, EOD1 or EOD3 (WO2015/022192, PCT/GB/2013/050072).

20 [60] A "transcription termination and polyadenylation region" as used herein is a sequence that drives the cleavage of the nascent RNA, whereafter a poly(A) tail is added at the resulting RNA 3' end, functional in plant cells. Transcription termination and polyadenylation signals functional in plant cells include, but are not limited to, 3'nos, 3'35S, 3'his and 3'g7.

[61] The term "protein" interchangeably used with the term "polypeptide" as used herein describes a group of
 25 molecules consisting of more than 30 amino acids, whereas the term "peptide" describes molecules consisting of up to 30 amino acids. Proteins and peptides may further form dimers, trimers and higher oligomers, i.e. consisting of more than one (poly)peptide molecule. Protein or peptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. The terms "protein" and "peptide" also refer to naturally modified proteins
 30 or peptides wherein the modification is effected e.g. by glycosylation, acetylation, phosphorylation and the like. Such modifications are well known in the art.

[62] The term "heterologous" refers to the relationship between two or more nucleic acid or protein sequences that are derived from different sources. For example, a promoter is heterologous with respect to an operably linked DNA region, such as a coding sequence if such a combination is not normally found in nature. In addition, a
 35 particular sequence may be "heterologous" with respect to a cell or organism into which it is inserted (i.e. does not

naturally occur in that particular cell or organism).

[63] The term "recombinant gene" refers to any gene that contains: a) DNA sequences, including regulatory and coding sequences that are not found together in nature, or b) sequences encoding parts of proteins not naturally adjoined, or c) parts of promoters that are not naturally adjoined. Accordingly, a recombinant gene may
5 comprise regulatory sequences and coding sequences that are derived from different sources, or comprise regulatory sequences, and coding sequences derived from the same source, but arranged in a manner different from that found in nature.

[64] Any of the promoters and heterologous nucleic acid sequences described above may be provided in a recombinant vector. A recombinant vector typically comprises, in a 5' to 3' orientation: a promoter to direct the
10 transcription of a nucleic acid sequence and a nucleic acid sequence. The recombinant vector may further comprise a 3' transcriptional terminator, a 3' polyadenylation signal, other untranslated nucleic acid sequences, transit and targeting nucleic acid sequences, selectable markers, enhancers, and operators, as desired. The wording "5' UTR" refers to the untranslated region of DNA upstream, or 5' of the coding region of a gene and "3' UTR" refers to the untranslated region of DNA downstream, or 3' of the coding region of a gene. Means for preparing recombinant
15 vectors are well known in the art. Methods for making recombinant vectors particularly suited to plant transformation are described in US4971908, US4940835, US4769061 and US4757011. Typical vectors useful for expression of nucleic acids in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens*. One or more additional promoters may also be provided in the recombinant vector. These promoters may be operably linked, for example, without limitation, to any of the nucleic acid
20 sequences described above. Alternatively, the promoters may be operably linked to other nucleic acid sequences, such as those encoding transit peptides, selectable marker proteins, or antisense sequences. These additional promoters may be selected on the basis of the cell type into which the vector will be inserted. Also, promoters which function in bacteria, yeast, and plants are all well taught in the art. The additional promoters may also be selected on the basis of their regulatory features. Examples of such features include enhancement of transcriptional activity,
25 inducibility, tissue specificity, and developmental stage-specificity.

[65] The recombinant vector may also contain one or more additional nucleic acid sequences. These additional nucleic acid sequences may generally be any sequences suitable for use in a recombinant vector. Such nucleic acid sequences include, without limitation, any of the nucleic acid sequences, and modified forms thereof,
30 described above. The additional structural nucleic acid sequences may also be operably linked to any of the above described promoters. The one or more structural nucleic acid sequences may each be operably linked to separate promoters. Alternatively, the structural nucleic acid sequences may be operably linked to a single promoter (i.e., a single operon).

[66] Yet another embodiment provides a host cell, such as an *E. coli* cell, an *Agrobacterium* cell, a yeast cell, an algal cell, or a plant cell, comprising the isolated nucleic acid according to the invention, or the recombinant gene
35 according to the invention.

[67] Other nucleic acid sequences may also be introduced into the host cell along with the promoter and structural nucleic acid sequence, e. g. also in connection with the vector of the invention. These other sequences may include 3' transcriptional terminators, 3' polyadenylation signals, other untranslated nucleic acid sequences, transit or targeting sequences, selectable markers, enhancers, and operators. Preferred nucleic acid sequences of the present invention, including recombinant vectors, structural nucleic acid sequences, promoters, and other regulatory elements, are described above.

[68] In further embodiments, a plant and a plant cell are provided comprising the recombinant gene according to the invention. Yet a further embodiment provides seeds obtainable from the plant according to the invention. In another embodiment, the plants or seeds according to the invention are seed crop plants or seeds.

[69] The plant cell or plant comprising the recombinant gene according to the invention can be a plant cell or a plant comprising a recombinant gene of which either the promoter, or the heterologous nucleic acid sequence operably linked to said promoter, are heterologous with respect to the plant cell. Such plant cells or plants may be transgenic plant in which the recombinant gene is introduced via transformation. Alternatively, the plant cell or plant may comprise the promoter according to the invention derived from the same species operably linked to a nucleic acid which is also derived from the same species, i.e. neither the promoter nor the operably linked nucleic acid is heterologous with respect to the plant cell, but the promoter is operably linked to a nucleic acid to which it is not linked in nature. A recombinant gene can be introduced in the plant or plant cell via transformation, such that both the promoter and the operably linked nucleotide are at a position in the genome in which they do not occur naturally. Alternatively, the promoter according to the invention can be integrated in a targeted manner in the genome of the plant or plant cell upstream of an endogenous nucleic acid encoding an expression product of interest, i.e. to modulate the expression pattern of an endogenous gene. The promoter that is integrated in a targeted manner upstream of an endogenous nucleic acid can be integrated in cells of a plant species from which it is originally derived, or in cells of a heterologous plant species. Alternatively, a heterologous nucleic acid can be integrated in a targeted manner in the genome of the plant or plant cell downstream of the promoter according to the invention, such that said heterologous nucleic acid is expressed seed-preferentially. Said heterologous nucleic acid is a nucleic acid which is heterologous with respect to the promoter, i.e. the combination of the promoter with said heterologous nucleic acid is not normally found in nature. Said heterologous nucleic acid may be a nucleic acid which is heterologous to said plant species in which it is inserted, but it may also naturally occur in said plant species at a different location in the plant genome. Said promoter or said heterologous nucleic acid can be integrated in a targeted manner in the plant genome via targeted sequence insertion, using, for example, the methods as described in WO2005/049842.

[70] Plants comprising at least two recombinant genes according to the invention wherein the nucleic acid comprising seed- and funiculus-preferential promoter activity is different in each recombinant gene are, for example, plants comprising a first recombinant gene comprising a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 3 or a functional fragment thereof, and a second recombinant gene comprising a nucleotide sequence having at least 95% sequence identity to any one of SEQ ID NO: 4 to SEQ ID NO: 7 or a functional

fragment thereof. It will be clear that, when the first recombinant gene comprises a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: x or a functional fragment thereof, wherein SEQ ID NO: x is selected from any one of SEQ ID NO: 3 to SEQ ID NO: 7, the second recombinant gene may comprise a nucleotide sequence having at least 95% sequence identity to any one of the sequences according to the invention or a functional fragment thereof, except to SEQ ID NO: x. Said plants are suitable to express different genes with the same tissue-specificity, however without the negative features associated with the repeated use of one promoter, such as gene silencing or recombination of a vector comprising the recombinant genes. The at least two recombinant genes according to the invention may be present at one locus in the genome of said plant, and may be derived from the same transforming DNA molecule.

10 [71] Plants according to the invention may comprise one or more recombinant genes according to the invention, but may in addition contain a recombinant gene comprising a nucleic acid comprising promoter activity which is preferential or specific to other plant tissues, such as apical meristem, flower buds, cotyledons, flowers, pods, roots, and leaves, operably linked to a nucleic acid sequence encoding an expression product of interest. The recombinant gene according to the invention and the recombinant gene comprising a nucleic acid comprising another promoter activity may be present at one locus and may be derived from the same transforming DNA molecule.

[72] Yet another embodiment provides a method of producing a transgenic plant comprising the steps of (a) introducing or providing the recombinant gene according to the invention to a plant cell to create transgenic cells; and (b) regenerating transgenic plants from said transgenic cell.

20 [73] "Introducing" in connection with the present application relates to the placing of genetic information in a plant cell or plant by artificial means. This can be effected by any method known in the art for introducing RNA or DNA into plant cells, protoplasts, calli, roots, tubers, seeds, stems, leaves, seedlings, embryos, pollen and microspores, other plant tissues, or whole plants. "Introducing" also comprises stably integrating into the plant's genome. Introducing the recombinant gene can be performed by transformation.

25 [74] The term "transformation" herein refers to the introduction (or transfer) of nucleic acid into a recipient host such as a plant or any plant parts or tissues including plant cells, protoplasts, calli, roots, tubers, seeds, stems, leaves, seedlings, embryos and pollen. Plants containing the transformed nucleic acid sequence are referred to as "transgenic plants". Transformed, transgenic and recombinant refer to a host organism such as a plant into which a heterologous nucleic acid molecule (e.g. an expression cassette or a recombinant vector) has been introduced. The nucleic acid can be stably integrated into the genome of the plant.

[75] As used herein, the phrase "transgenic plant" refers to a plant having an introduced nucleic acid stably introduced into a genome of the plant, for example, the nuclear or plastid genomes. In other words, plants containing transformed nucleic acid sequence are referred to as "transgenic plants". Transgenic and recombinant refer to a host organism such as a plant into which a heterologous nucleic acid molecule (e.g. the promoter, the

chimeric gene or the vector as described herein) has been introduced. The nucleic acid can be stably integrated into the genome of the plant.

[76] Transformation methods are well known in the art and include *Agrobacterium*-mediated transformation. *Agrobacterium*-mediated transformation of cotton has been described e.g. in US patent 5,004,863, in US patent 5 6,483,013 and WO2000/71733. Plants may also be transformed by particle bombardment: Particles of gold or tungsten are coated with DNA and then shot into young plant cells or plant embryos. This method also allows transformation of plant plastids. Viral transformation (transduction) may be used for transient or stable expression of a gene, depending on the nature of the virus genome. The desired genetic material is packaged into a suitable plant virus and the modified virus is allowed to infect the plant. The progeny of the infected plants is virus free and also free of the inserted gene. Suitable methods for viral transformation are described or further detailed e. g. in WO 10 90/12107, WO 03/052108 or WO 2005/098004. Further suitable methods well-known in the art are microinjection, electroporation of intact cells, polyethyleneglycol-mediated protoplast transformation, electroporation of protoplasts, liposome-mediated transformation, silicon-whiskers mediated transformation etc. Said transgene may be stably integrated into the genome of said plant cell, resulting in a transformed plant cell. The transformed plant cells 15 obtained in this way may then be regenerated into mature fertile transformed plants.

[77] Further provided is a method of effecting seed- and funiculus-preferential expression of a nucleic acid comprising introducing the recombinant gene according to the invention into the genome of a plant, or providing the plant according to the invention. Also provided is a method for altering seed properties of a plant or to produce a commercially relevant product in a plant, comprising introducing the recombinant gene according to the invention 20 into the genome of a plant, or providing the plant according to the invention. In another embodiment, said plant is a seed crop plant.

[78] "Seed properties" as used herein are properties of the seed. Seed properties can, for example, be seed yield, seed storage compound production, seed compound accumulation, seed nutrient accumulation; seed micronutrient accumulation; seed storage compound quality, seed compound composition, seed quality, biotic stress tolerance such as disease tolerance, abiotic stress tolerance, herbicide tolerance, seed dormancy, seed imbibition, seed germination, seed vigor. Seed storage compounds can, for example, be, seed oil, seed starch, or seed protein. 25

[79] Seed properties may be modulated by modulating metabolic pathways, such as starch metabolism, sugar metabolism, inositol phosphate metabolism, glycolysis, amino acid biosynthesis, carbon metabolism, nucleotide metabolism, oxidative pentose phosphate cycle, fatty acid biosynthesis, protein synthesis, or phytate metabolism, and modulating secondary metabolism pathways. Another example is the methyl recycling metabolic activity impacting chromatin remodeling, phospholipid biosynthesis and cell wall lignification. Such metabolic pathways can be modulated by, for example, overexpressing or down-regulating a gene involved in one or more of the metabolic pathways using the seed- and funiculus-preferential promoter according to the invention. 30

[80] Yield as used herein can comprise yield of the plant or plant part which is harvested, such as seed, including seed oil content, seed protein content, seed weight, seed number. Increased yield can be increased yield per plant, and increased yield per surface unit of cultivated land, such as yield per hectare. Yield can be increased by modulating, for example, by increasing seed size or oil content or indirectly by increasing the tolerance to biotic and abiotic stress conditions and decreasing seed abortion.

[81] Quality as used herein can comprise quality of the seed or grain such as beneficial carbohydrate composition or level, beneficial amino acid composition or level, beneficial fatty acid composition or level, nutritional value, seed and fiber content.

[82] Abiotic stress tolerance as used herein can comprise resistance to environmental stress factors such as drought, extreme (high or low) temperatures.

[83] Biotic stress tolerance as used herein can comprise pest resistance, such as resistance to fungal, bacterial, bacterial or viral pathogens or insects.

[84] Also provided is the use of the isolated nucleic acid according to the invention to regulate expression of an operably linked nucleic acid in a plant, and the use of the isolated nucleic acid according to the invention, or the recombinant gene according to the invention to alter seed properties of a plant or to produce a commercially relevant product in a plant. In a further embodiment, said plant is a trait as used herein refers to beneficial properties of the plant, such as commercially beneficial properties of a plant.

[85] Also provided is the use of the isolated nucleic acid according to the invention to identify other nucleic acids comprising seed- and funiculus-preferential promoter activity.

[86] The promoters according to the invention can further be used to create hybrid promoters, i.e. promoters containing (parts of) one or more of the promoters(s) of the current invention and (parts of) other promoter which can be newly identified or known in the art. Such hybrid promoters may have optimized tissue specificity or expression level.

[87] Yet another embodiment provides a method of producing food, feed, or an industrial product comprising (a) obtaining the plant or a part thereof, according to the invention; and (b) preparing the food, feed or industrial product from the plant or part thereof. In another embodiment, 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.

[88] A "seed crop" or "seed crop plant" as used herein is a crop grown for its seeds or material derived from the seeds. Examples of seed crops are rice, maize, wheat, barley, millet, rye, oats, camelina, crambe, *Linum*, castor bean, calendula, safflower, sunflower, soybean, cotton, or *Brassica* species, such as *Brassica napus*, *Brassica juncea*, *Brassica carinata*, *Brassica rapa*, *Brassica oleracea*, and *Brassica nigra*.

[89] "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*;
 5 *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*; *Diploaxis* species; *Lepidium* species; *Nasturtium* species; *Orychophragmus* species; *Armoracia* species, *Eutrema* species; *Lepidium* species; and *Arabidopsis* species.

[90] Said Brassicaceae plant can be a *Brassica* plant. "Brassica plant" refers to allotetraploid or amphidiploid
 10 *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).

[91] Crop plants of the *Brassica* species are, for example, *Brassica napus*, *Brassica juncea*, *Brassica carinata*, *Brassica rapa* (syn. *B. campestris*), *Brassica oleracea* or *Brassica nigra*.

[92] The plants according to the invention may additionally contain an endogenous or a transgene, which
 15 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 [EP 0 508 909 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
 20 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 thioesterase increase to obtain high laurate, which confers pollination control, such as such as barnase under
 25 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.

[93] The plants or seeds of the plants 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,
 30 Quinmerac, Quizalofop, Tepraloxymid, 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, Chloromequat-chloride, Coniothyrium minitans, Cyproconazole, Cyprodinil, Difenconazole, Dimethomorph, Dimoxystrobin, Epoxiconazole, Famoxadone, Fluazinam, Fludioxonil,
 35 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-trifluoroethyl)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.

[94] Whenever reference to a "plant" or "plants" according to the invention is made, it is understood that also plant parts (cells, tissues or organs, seed pods, seeds, severed parts such as roots, leaves, flowers, pollen, etc.), progeny of the plants which retain the distinguishing characteristics of the parents, such as seed obtained by selfing or crossing, e.g. hybrid seed (obtained by crossing two inbred parental lines), hybrid plants and plant parts derived there from are encompassed herein, unless otherwise indicated.

[95] In some embodiments, the plant cells of the invention as well as plant cells generated according to the methods of the invention, may be non-propagating cells.

[96] The obtained plants according to the invention can be used in a conventional breeding scheme to produce more plants with the same characteristics or to introduce the same characteristic 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.

[97] "Creating propagating material", as used herein, relates to any means known 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).

[98] As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a nucleic acid which is functionally or structurally defined, may comprise additional DNA regions etc.

[99] Furthermore, the disclosed invention is expected to yield similar results in other seed crop plant species. Particularly, it is expected to drive seed- and funiculus-preferential expression in soybean. It is also expected to drive seed- and funiculus-preferential expression in wheat. The disclosed promoter may lead to a seed- and funiculus-preferential expression in cotton.

[100] The sequence listing contained in the file named „BCS16-2003_ST25.txt“, which is 45 kilobytes (size as measured in Microsoft Windows®), contains 24 sequences SEQ ID NO: 1 through SEQ ID NO: 24 is filed herewith by electronic submission and is incorporated by reference herein.

[101] In the description and examples, reference is made to the following sequences:

15 SEQUENCES

- SEQ ID NO: 1: nucleotide sequence of the T-DNA Pslp1 BnA::GUS.
- SEQ ID NO: 2: nucleotide sequence of the T-DNA Pslp1 intron BnA::GUS.
- SEQ ID NO: 3: nucleotide sequence of the promoter region Pslp1 intron BnA.
- SEQ ID NO: 4: nucleotide sequence of the promoter region Pslp1 intron BnC.
- 20 SEQ ID NO: 5: nucleotide sequence of the promoter region Pslp1 intron Br.
- SEQ ID NO: 6: nucleotide sequence of the promoter region Pslp1 intron Bo.
- SEQ ID NO: 7: nucleotide sequence of the promoter region Pslp1 intron BjA.
- SEQ ID NO: 8: amino acid sequence of SLP1 BnA.
- SEQ ID NO: 9: amino acid sequence of SLP1 BnC.
- 25 SEQ ID NO: 10: amino acid sequence of SLP1 Br.
- SEQ ID NO: 11: amino acid sequence of SLP1 Bo.
- SEQ ID NO: 12: amino acid sequence of SLP1 BjA.
- SEQ ID NO: 13: nucleotide sequence of the coding sequence of SLP1 BnA.
- SEQ ID NO: 14: nucleotide sequence of the coding sequence of SLP1 BnC.
- 30 SEQ ID NO: 15: nucleotide sequence of the coding sequence of SLP1 Br.
- SEQ ID NO: 16: nucleotide sequence of the coding sequence of SLP1 Bo.
- SEQ ID NO: 17: nucleotide sequence of the coding sequence of SLP1 BjA.
- SEQ ID NO: 18: Promoter consensus sequence.
- SEQ ID NO: 19: UTR consensus sequence 1.

SEQ ID NO: 20: UTR consensus sequence 2.

SEQ ID NO: 21: UTR consensus sequence 3.

SEQ ID NO: 22: Intron consensus sequence 1.

SEQ ID NO: 23: Intron consensus sequence 2.

5 SEQ ID NO: 24: Intron consensus sequence 3.

EXAMPLES

[102] Unless stated otherwise in the Examples, all recombinant DNA techniques are carried out according to standard protocols as described in Sambrook and Russell (2001) *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, NY, in Volumes 1 and 2 of Ausubel et al. (1994) *Current Protocols in Molecular Biology*, Current Protocols, USA and in Volumes I and II of Brown (1998) *Molecular Biology LabFax*, Second Edition, Academic Press (UK). Standard materials and methods for plant molecular work are described in *Plant Molecular Biology Labfax* (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK. Standard materials and methods for polymerase chain reactions can be found in Dieffenbach and Dveksler (1995) *PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, and in McPherson et al. (2000) *PCR - Basics: From Background to Bench*, First Edition, Springer Verlag, Germany.

Example 1 - Generation of expression constructs with the Pslp1BnA promoter fragments of *Brassica napus* operably linked to the GUS reporter gene (Pslp1 BnA::GUS and Pslp1 intron BnA::GUS)

[103] Because the SLP1 gene structure contains an intron immediately after the translation start (Figure 1), it was hypothesized that this intron may be required for the promoter activity. Therefore expression constructs with or without this first intron were generated.

[104] The promoter sequence of the *Brassica napus* Pslp1 BnA promoter (position 1 to 1414 of SEQ ID NO: 3 or 5' to 3' position 139 to 1552 of SEQ ID NO:1) isolated from an *in house* developed *Brassica napus* line, the GUS gene (β -glucuronidase) with intron (5' to 3' position 1553 to 3553 of SEQ ID NO: 1) and a fragment of the 3' untranslated region (UTR) of the TL-DNA gene 7 of *Agrobacterium tumefaciens* octopine (5' to 3' position 3610 to 3813 of SEQ ID NO: 1) were assembled in a vector which contains the bar selectable marker cassette (position 3894 to 6404 of SEQ ID NO: 1) to result in the T-DNA Pslp1 BnA::GUS (SEQ ID NO: 1).

[105] The promoter sequence of the *Brassica napus* slp1BnA promoter plus the first intron (SEQ ID NO: 3 or 5' to 3' position 139 to 2193 of SEQ ID NO: 2) isolated from an *in house* developed *Brassica napus* line, the GUS gene (β -glucuronidase) with intron (5' to 3' position 2194 to 4191 of SEQ ID NO: 2) and a fragment of the 3' untranslated region (UTR) of the TL-DNA gene 7 of *Agrobacterium tumefaciens* octopine (5' to 3' position 4248 to 4451 of SEQ ID NO: 2) were assembled in a vector which contains the bar selectable marker cassette (position 4532 to 7042 of SEQ ID NO: 2) to result in the T-DNA Pslp1 intron BnA::GUS (SEQ ID NO: 2).

Example 2 - Generation of transgenic plants comprising the Pslp1 BnA::GUS or Pslp1 intron BnA::GUS

[106] In a next step the recombinant vectors comprising the expression cassettes of example 1, i. e. Pslp1 BnA::GUS and Pslp1 intron BnA::GUS, were used to stably transform *Brassica napus*.

Example 3 - *In planta* expression pattern of Pslp1BnA::GUS and Pslp1BnAintron::GUS in *Brassica napus*

5 [107] The *in planta* expression pattern of Pslp1 BnA::GUS and Pslp1 intron BnA::GUS in the different tissues of *Brassica napus* seeds was monitored according to the method of Jasik et al. 2011.

[108] Figure 2 provides the semi quantitative assessment of the GUS labelling in the seed coat and the embryo of transgenic lines carrying either the Pslp1 BnA::GUS or Pslp1 intron BnA::GUS T-DNAs. For both constructs, the GUS staining is detected in both embryos and seed coat. For both constructs, the intensity of the staining in the embryo increases as the embryo progresses through the developmental stages, in other words, the expression level is higher at late stage than at early stage. In contrast, in the seed coat, the labelling is the strongest at early stages and then decreases as it progresses towards maturation. Though both constructs confer the same expression pattern, lines carrying the T-DNA Pslp1 intron BnA::GUS have a moderate expression level while the lines carrying the T-DNA Pslp1BnA::GUS have a weak expression level. The presence of the intron after the translation start ATG increases the expression level by at least 3 fold, up to more than 10 fold.

10
15

[109] Figure 3 shows the GUS labelling of the reporter gene in the endosperm and seed coat at early (panels A and B) and late (panel C) seed developmental stages. At early stage, both endosperm and seed coat are labelled, with about the same intensity. The close-up view shows that the outer integument of the seed coat is more intensely stained than the inner integument. The activity of the Pslp1 intron BnA promoter fragment is moderate in the endosperm and seed coat at early stage. The close up view on the seed coat and endosperm at late stage show that the GUS labelling occurs in the outer integument but not in the inner integument, nor in the endosperm residues. Figure 3 further shows the GUS labelling of the reporter gene in the funiculus. The same result was obtained with the lines carrying the Pslp1 BnA::GUS T-DNA though with a weaker intensity.

20

[110] Some GUS activity was also detected in the assessed non-seed tissues, namely stem, young pod, flowers (receptacle and peduncle) and leaves, though to a lower level, at least 2 fold weaker than in the seed and the funiculus tissues, thereby confirming the seed- and funiculus-preference of the promoter Pslp1 intron BnA.

25

Example 4 - Identification of the second *Brassica napus* copy of SLP1 and of the orthologues of SLP1 in *Brassica rapa*, *Brassica oleracea* and *Brassica juncea*

[111] The sequences of the second *Brassica napus* copy of SLP1 as well as the orthologues in *Brassica rapa*, *Brassica oleracea* and *Brassica juncea* were obtained by blasting the coding sequence of the SLP1 BnA against an *in-house* database of *Brassica napus*, *Brassica rapa*, *Brassica oleracea* and *Brassica juncea* sequences.

30

[112] The nucleotide sequences obtained in this way are given in SEQ ID NO: 13 to SEQ ID NO: 17. These nucleotide sequences were translated into amino acid sequences, given respectively in SEQ ID NO: 8 to SEQ ID NO: 12.

[113] In *Brassica rapa*, the result indicated that 2 copies of SLP1 are present in the genome, on two different chromosomes. As the nucleotide sequences of the two genomic fragments comprising the promoter and the genes are 100% identical, the nucleotide and amino acid sequences are provided only once. In *Brassica juncea*, no start codon was identified for the B copy of SLP1. This copy was therefore considered a pseudogene and was not further studied.

[114] Figure 6 shows the alignment of the retrieved amino acid sequence. Any two of these sequences share at least 97% sequence identity.

Examples 5 - RNA isolation from different *Brassica* tissues

[115] The following tissues were isolated from *Brassica napus*:

- a. Apical meristem 33 days after sowing (DAS) (including smallest leaves) (AM33)
- b. Big flower buds (> 5 mm) 42 DAS (BFB42)
- 15 c. Cotyledons (with hypocotyl) 10 DAS (CTYL10)
- d. Open flowers 52 DAS (OF52)
- e. Pods 14-20 DAS (Pod2)
- f. Pods 21-25 DAS (Pod3)
- g. Roots 14 DAS (Ro2w)
- 20 h. Small flower buds ≤ 5 mm 42 DAS (SFB42)
- i. Seeds 14-20 days after flowering (DAF) (Seed2)
- j. Seeds 21-25 DAF (Seed3)
- k. Seeds 26-30 DAF (Seed4)
- l. Seeds 31-35 DAF (Seed5)
- 25 m. Seeds 42 DAF (Seed6)
- n. Seeds 49 DAF (Seed7)
- o. Stem 14 DAS (St2w)
- p. Stem 33 DAS (St5w)
- q. Young leaf 33 DAS (≤ 3 cm leaf next to apical meristem) (YL33)

30

[116] The following tissues were isolated from *Brassica juncea*:

- a. Apical meristem 22 days after sowing (DAS) (including smallest leaves) (AM22)
- b. Big flower buds (> 5 mm) 35 DAS (BFB35)
- c. Cotyledons (with hypocotyl) 8 DAS (CTYL8)
- 35 d. Open flowers 35 DAS (OF35)

- e. Pods 14-20 DAS (Pod2)
- f. Pods 21-25 DAS (Pod3)
- g. Pods 26-30 DAS (Pod4)
- h. Pods 31-35 DAS (Pod5)
- 5 i. Roots 14 DAS (Ro2w)
- j. Small flower buds ≤ 5 mm 35 DAS (SFB35)
- k. Seeds 14-20 days after flowering (DAF) (Seed2)
- l. Seeds 21-25 DAF (Seed3)
- m. Seeds 26-30 DAF (Seed4)
- 10 n. Seeds 31-35 DAF (Seed5)
- o. Seeds 42 DAF (Seed6)
- p. Seeds 49 DAF (Seed7)
- q. Stem 14 DAS (St2w)
- r. Stem 22 DAS (St3w)
- 15 s. Young leaf 22 DAS (≤ 3 cm leaf next to apical meristem) (YL22)
- t. Old leaf 22 DAS (OL22)

[117] Total RNA from the different tissues was isolated according to standard methods.

[118] In our growth conditions, the correspondence between embryo developmental stages and the selected
20 time points is as follows:

- a. Between 10 and 13 DAF: torpedo stage
- b. Seed2 or between 14 and 20 DAF: "walking stick" cotyledon stage
- c. Seed3 or between 21 and 25 DAF: curled cotyledon stage
- d. Seed4 and Seed5 or between 26 and 35 DAF: green cotyledon stage
- 25 e. Seed6 and Seed7 or after 36 DAF: mature embryo

Example 6 - *In silico* expression analyses of the different copies of SLP1 of *Brassica napus* and their orthologues in *Brassica rapa*, *Brassica oleracea* and *Brassica juncea*

[119] Figure 5 shows the relative expression levels of the endogenous transcripts of the different *Brassica napus* (A and B), *Brassica oleracea* (C), *Brassica rapa* (D) and *Brassica juncea* (E) copies of SLP1 in different tissues, as
30 isolated in Example 5.

[120] The SLP1 BnA transcript (panel A) is clearly detected in the seed tissues (Seed2 to Seed7) and only barely detectable in other tissues. This result confirms, as determined *in planta*, that Pslp1 intron BnA has seed-preferential promoter activity.

[121] The SLP1 BnC transcript (panel B) is clearly detected in the seed tissues (Seed2 to Seed7) and only barely detectable in the other tissues. This result confirms, as determined *in planta*, that Pslp1 intron BnC has seed-preferential promoter activity.

5 [122] The SLP1 Br transcript (panel C) is clearly detected in the seed tissues (Seed2 to Seed7) and only barely detectable in the other tissues. This result confirms, as determined *in planta*, that Pslp1 intron Br has seed-preferential promoter activity.

[123] The SLP1 Bo transcript (panel D) is clearly detected in the seed tissues (Seed2 to Seed7) and only barely detectable in the other tissues. This result confirms, as determined *in planta*, that Pslp1 intron Bo has seed-preferential promoter activity.

10 [124] The SLP1 BjA transcript (panel E) is clearly detected in the seed tissues (Seed2, Seed4, Seed6 and Seed7) and only barely detectable in the other tissues. This result confirms, as determined *in planta*, that Pslp1 intron BjA has seed-preferential promoter activity.

Example 7 - Sequence analysis of the promoters and first intron of the SLP1 genes from *Brassica rapa*, *Brassica juncea*, *Brassica oleracea* and *Brassica napus*

15 [125] For the different SLP1 gene identified, the genomic DNA sequence upstream of the translation start and including the first intron was retrieved from an *in-house* database of *Brassica napus*, *Brassica rapa*, *Brassica oleracea* and *Brassica juncea* sequences. The nucleotide sequences obtained in this way are given in SEQ ID NO: 4 to SEQ ID NO: 7.

20 [126] Figure 6 shows the alignment of the about 400 bp sequence upstream of the translation start of the promoter sequences (SEQ ID NO: 3 to SEQ ID NO: 7). These promoters share a surprisingly high level of conservation in this region. Four consensus sequences were identified. The promoters described herein each comprise the following consensus sequence:

- a. Promoter consensus sequence is given in SEQ ID NO: 18;
- b. UTR consensus sequence 1 is given in SEQ ID NO: 19;
- 25 c. UTR consensus sequence 2 is given in SEQ ID NO: 20;
- d. UTR consensus sequence 3 is given in SEQ ID NO: 21.

[127] The presence of these consensus sequences in all analyzed promoter sequences described herein indicate that these consensus sequences are required for the observed seed- and funiculus-preferential expression pattern.

30 [128] Consequently, as Pslp1 BnC (position 1 to 1364 of SEQ ID NO: 4) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 intron BnC (SEQ ID NO: 4) sequence comprises the promoter consensus

sequence and the 3 UTR consensus sequences, it can also be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 Br (position 1 to 1365 of SEQ ID NO: 5) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can also be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 intron Br (SEQ ID NO: 5) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can also be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 Bo (position 1 to 1358 of SEQ ID NO: 6) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 intron Bo (SEQ ID NO: 6) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 BJA (position 1 to 1363 of SEQ ID NO: 7) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can be concluded that it has seed- and funiculus-preferential promoter activity. As Pslp1 intron BJA (SEQ ID NO: 7) sequence comprises the promoter consensus sequence and the 3 UTR consensus sequences, it can be concluded that it has seed- and funiculus-preferential promoter activity.

[129] More generally, these results indicate that a *Brassica* promoter comprising the promoter consensus sequence and the 3 UTR consensus sequences would have seed- and funiculus-preferential promoter activity.

[130] Figure 7 shows the alignment of the first about 300 bp of the intron sequence of the promoter sequences (SEQ ID NO: 3 to SEQ ID NO: 7). This intron shares a surprisingly high level of conservation in this region. Three consensus sequences were identified in this first intron. The promoters described herein each comprise the following consensus sequence:

- a. Intron consensus sequence 1 is given in SEQ ID NO: 22;
- b. Intron consensus sequence 2 is given in SEQ ID NO: 23;
- c. Intron consensus sequence 3 is given in SEQ ID NO: 24.

[131] The presence of these consensus sequences in all analyzed intron sequences described herein indicates that these consensus sequences may be required for the observed increased expression level compared to the expression level achieved without the first intron.

[132] Consequently, as the intron in Pslp1 intron BnC (position 1368 to 1650 of SEQ ID NO: 4) comprises the intron consensus sequences 1 to 3, it can be concluded that Pslp1 intron BnC has higher (at least 3 fold, up to 10 fold) seed- and funiculus-preferential promoter activity than Pslp1 BnC (position 1 to 1364 of SEQ ID NO: 4). As the intron in Pslp1 intron Br (position 1369 to 2001 of SEQ ID NO: 5) comprises the intron consensus sequences 1 to 3, it can be concluded that Pslp1 intron Br (SEQ ID NO: 5) has higher (at least 3 fold, up to 10 fold) seed- and funiculus-preferential promoter activity than Pslp1 Br (position 1 to 1365 of SEQ ID NO: 5). As the intron in Pslp1 intron Bo (position 1362 to 1647 of SEQ ID NO: 6) comprises the intron consensus sequences 1 to 3, it can be concluded that Pslp1 intron Bo (SEQ ID NO: 6) has higher (at least 3 fold, up to 10 fold) seed- and funiculus-preferential promoter activity than Pslp1 Bo (position 1 to 1358 of SEQ ID NO: 6). As the intron in Pslp1 intron BJA

(position 1367 to 2001 of SEQ ID NO: 7) comprises the intron consensus sequences 1 to 3, it can be concluded that Pslp1 intron BJA (SEQ ID NO: 7) has higher (at least 3 fold, up to 10 fold) seed- and funiculus-preferential promoter activity than Pslp1 BJA (position 1 to 1363 of SEQ ID NO: 7).

[133] More generally, these results indicate that a *Brassica* promoter comprising an intron comprising the intron consensus sequences 1 to 3 would have higher (at least 3 fold, up to 10 fold) promoter activity than the same *Brassica* promoter without such intron.

CLAIMS

1. An isolated nucleic acid having seed- and funiculus-preferential promoter activity selected from the group consisting of:
 - a. a nucleic acid comprising the nucleotide sequence selected from the group:
 - 5 i. the nucleotide sequence from position 1 to position 1414 of SEQ ID NO: 3,
 - ii. the nucleotide sequence from position 1 to position 1364 of SEQ ID NO: 4,
 - iii. the nucleotide sequence from position 1 to position 1365 of SEQ ID NO: 5,
 - iv. the nucleotide sequence from position 1 to position 1358 of SEQ ID NO: 6, and
 - v. the nucleotide sequence from position 1 to position 1363 of SEQ ID NO: 7,
 - 10 or a functional fragment thereof; and
 - b. a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of a., or a functional fragment thereof.

2. The nucleic acid according to claim 1 comprising:
 - a. the nucleotide sequence of SEQ ID NO: 18;
 - 15 b. the nucleotide sequence of SEQ ID NO: 19;
 - c. the nucleotide sequence of SEQ ID NO: 20; and
 - d. the nucleotide sequence of SEQ ID NO: 21.

3. The nucleic acid according to claim 1 or 2 comprising an intron, the nucleotide sequence of which is selected from the group of:
 - 20 a. a nucleotide sequence selected from the group:
 - i. the nucleotide sequence from position 1418 to position 2055 of SEQ ID NO: 3,
 - ii. the nucleotide sequence from position 1368 to position 1650 of SEQ ID NO: 4,
 - iii. the nucleotide sequence from position 1369 to position 2001 of SEQ ID NO: 5,
 - iv. the nucleotide sequence from position 1362 to position 1647 of SEQ ID NO: 6, and
 - 25 v. the nucleotide sequence from position 1367 to position 2001 of SEQ ID NO: 7,
 - or a functional fragment thereof; and
 - b. a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of a., or a functional fragment thereof.

4. The nucleic acid according to claim 3 comprising:
 - 30 a. the nucleotide sequence of SEQ ID NO: 22;
 - b. the nucleotide sequence of SEQ ID NO: 23; and
 - c. the nucleotide sequence of SEQ ID NO: 24in the intron sequence.

5. The nucleic acid according to claim 3 or 4 having higher seed- and funiculus-preferential promoter activity than the nucleic acid according to claim 1 or 2.
6. The isolated nucleic acid according to any one of claims 1 to 5 selected from the group:
 - a. a nucleic acid comprising any one of SEQ ID NOs: 3 to 7, or a functional fragment thereof, and
 - 5 b. a nucleic acid comprising a nucleotide sequence having at least 80% sequence identity to any one of SEQ ID NOs: 3 to 7, or a functional fragment thereof.
7. A recombinant gene comprising the nucleic acid according to any one of claims 1 to 6 operably linked to a heterologous nucleic acid sequence encoding an expression product of interest, and optionally a transcription termination and polyadenylation sequence, preferably a transcription termination and polyadenylation region
10 functional in plants.
8. The recombinant gene according to claim 7, wherein the expression product of interest is an RNA molecule capable of modulating the expression of a gene or is a protein.
9. A host cell comprising the isolated nucleic acid according to any one of claims 1 to 6, or the recombinant gene according to claim 7 or 8.
- 15 10. The host cell of claim 9 which is an *E. coli* cell, an *Agrobacterium* cell, an algal cell, a yeast cell, or a plant cell.
11. A plant comprising the recombinant gene of claim 7 or 8.
12. The plant according to claim 11, comprising at least two recombinant genes according to claim 7, wherein the nucleic acid according to any one of claims 1 to 6, and, optionally, the heterologous nucleic acid sequence, is different in each recombinant gene.
- 20 13. Seeds obtainable from the plant according to claim 11 or 12.
14. The plant or plant cell or seeds according to any one of claims 10 to 13, which is a seed crop plant, or a cell or plant part.
15. Method of producing a transgenic plant comprising the steps of:
 - a. introducing or providing the recombinant gene according to claim 7 or 8 to a plant cell to create
25 transgenic cells; and
 - b. regenerating transgenic plants from said transgenic cell.
16. Method of effecting seed- and funiculus-preferential expression of a nucleic acid comprising introducing the recombinant gene according to claim 7 or 8 into the genome of a plant, or providing the plant according to claim 11 or 12.

17. Method for altering seed properties of a plant or to produce a commercially relevant product in a plant, said method comprising introducing the recombinant gene according to claim 7 or 8 into the genome of a plant, or providing the plant according to claim 11 or 14.
18. Use of the isolated nucleic acid according to any one of claims 1 to 6 to regulate expression of an operably
5 linked nucleic acid in a plant.
19. Use of the isolated nucleic acid according to any one of claims 1 to 6, or the recombinant gene according to claim 7 or 8 to alter seed properties of a plant or to produce a commercially relevant product in a plant.
20. Use of the isolated nucleic acid according to any one of claims 1 to 6 to identify other nucleic acids comprising seed- and funiculus-preferential promoter activity.
- 10 21. The method according to any one of claims 15 to 17, or the use according to claims 18 or 19, wherein said plant is a seed crop plant.
22. A method of producing food, feed, or an industrial product comprising
- a) obtaining the plant or a part thereof, of any one of claims 11 to 14; and
 - b) preparing the food, feed or industrial product from the plant or part thereof.
- 15 23. The method of claim 22 wherein
- a. the food or feed is oil, meal, grain, starch, flour or protein; or
 - b. the industrial product is biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical.

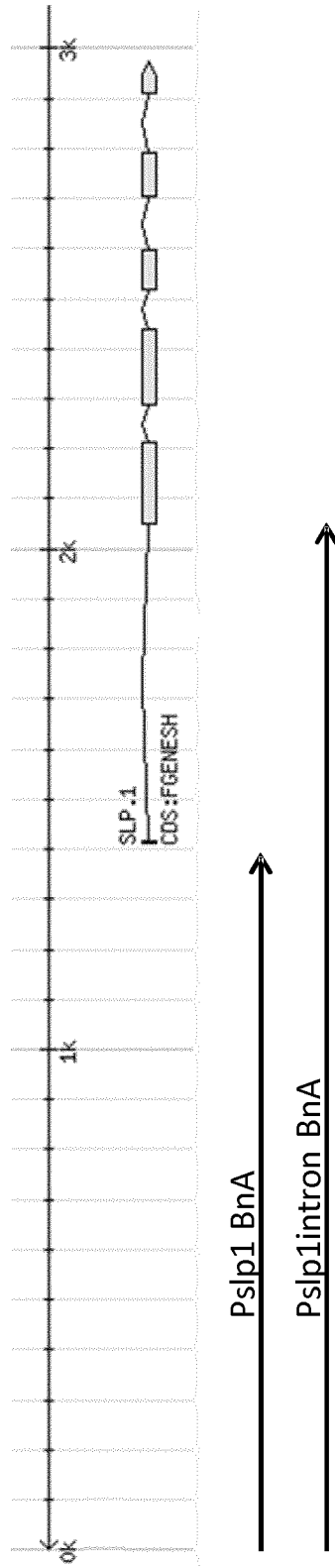


Figure 1

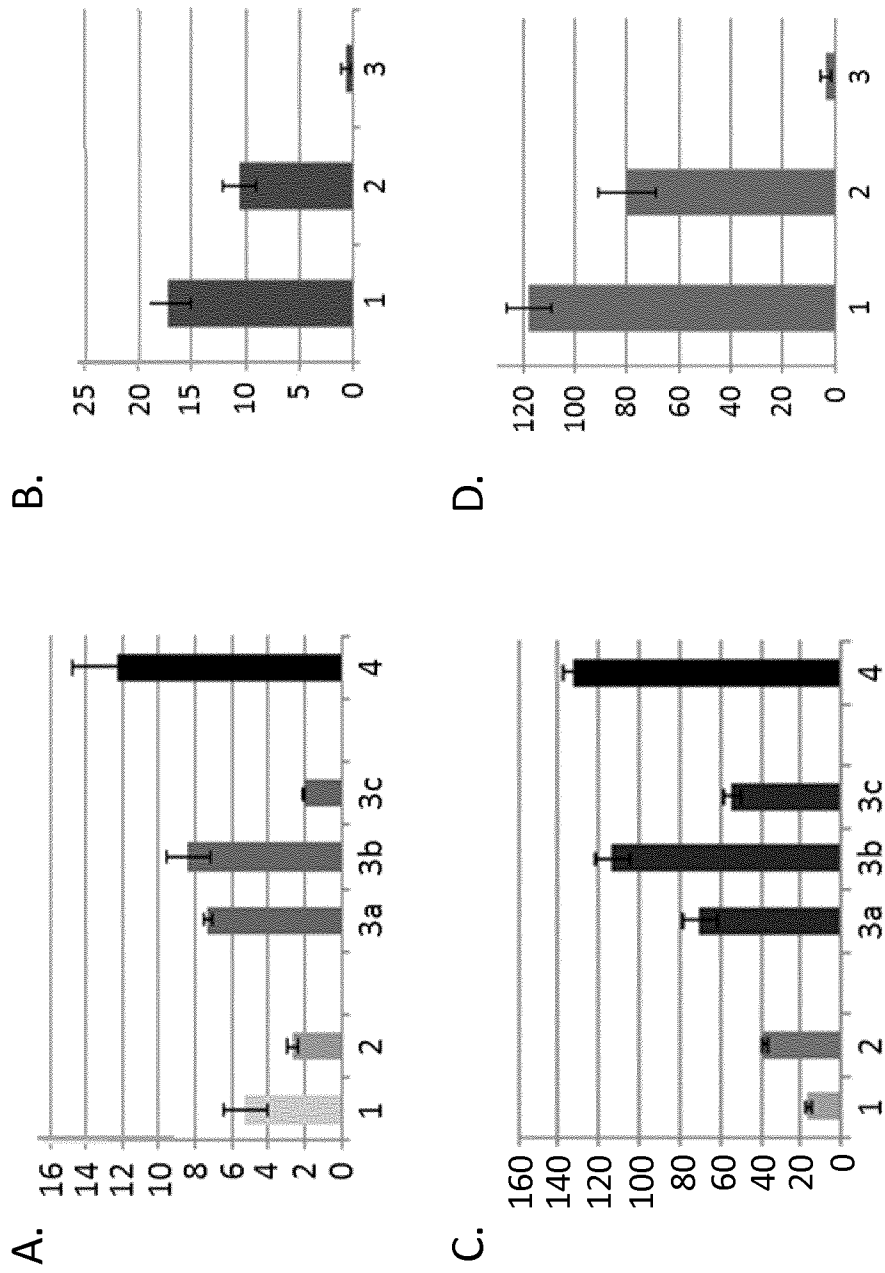


Figure 2

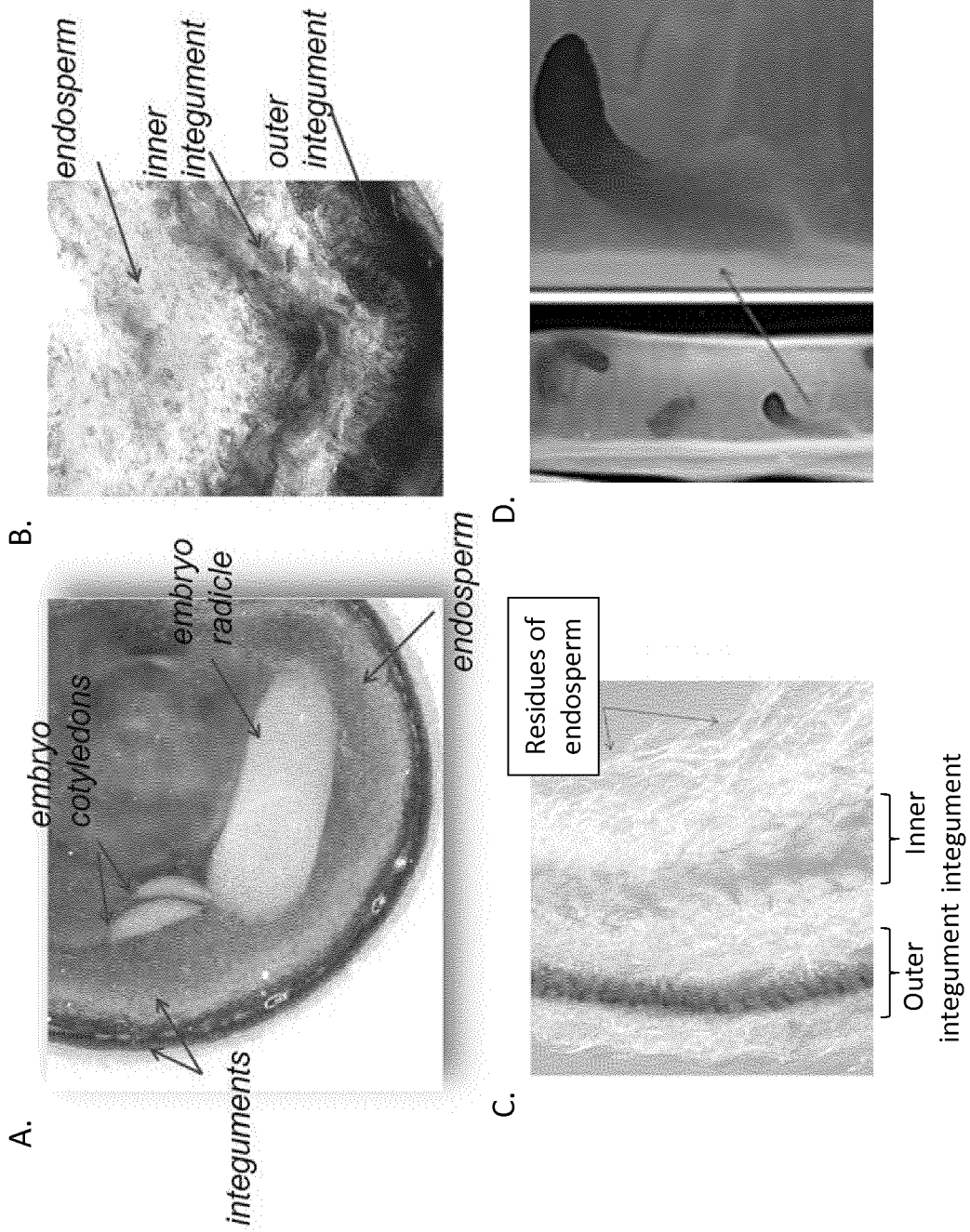


Figure 3

```

SEQ ID NO: 8 1 melppkvknlllldsegkriaaakyysgdwptntakeafekavfskqtknartevevtaleniiivykfv
SEQ ID NO: 9 1 melppkvknlllldsegkriaaakyysgdwptntakeafekavfskqtknartevevtaleniiivykfv
SEQ ID NO: 10 1 melppkvknlllldsegkriaaakyysgdwptntakeafekavfskqtknartevevtaleniiivykfv
SEQ ID NO: 12 1 melppkvknlllldsegkriaaakyysgdwptntakeafekavfskqtknartevevtaleniiivykfv
SEQ ID NO: 11 1 melppkvknlllldsegkriaaakyysgdwptntakeafekavfskqtknartevevtaleniiivykfv
*****:*****
71 qdlhffvtggeeeneelilasvlgglfdavnlllrgnadkrealnldlflcfdeiiddggivletdanvi
71 qdlhffvtggeeeneelivasl--lfdavnlllrgnadkrealnldlflcfdeiiddggivletdanvi
71 qdlhffvtggeeeneelivasvlgglfdavnlllrgnvdkrealnldlflcfdeiiddggivletdanvi
71 qdlhffvtggeeeneelivasvlgglfdavnlllrgnvdkrealnldlflcfdeiiddggivletdanvi
71 qdlhffvsggeeeneelivasvlgglfdavnlllrgnadkrealnldlflcfdeiiddggivletdanvi
*****:*****:***:* *****
141 aekaginsidpnaplseqtisqalatarehltrslmk
139 aekaginsidpnaplseqtisqalatarehltrslmk
141 aekaginsidpnaplseqtisqalatarehltrslmk
141 aekaginsidpnaplseqtisqalatarehltrslmk
141 aekaginsidpnaplseqtisqalatarehltrslmk
*****

```

Figure 4

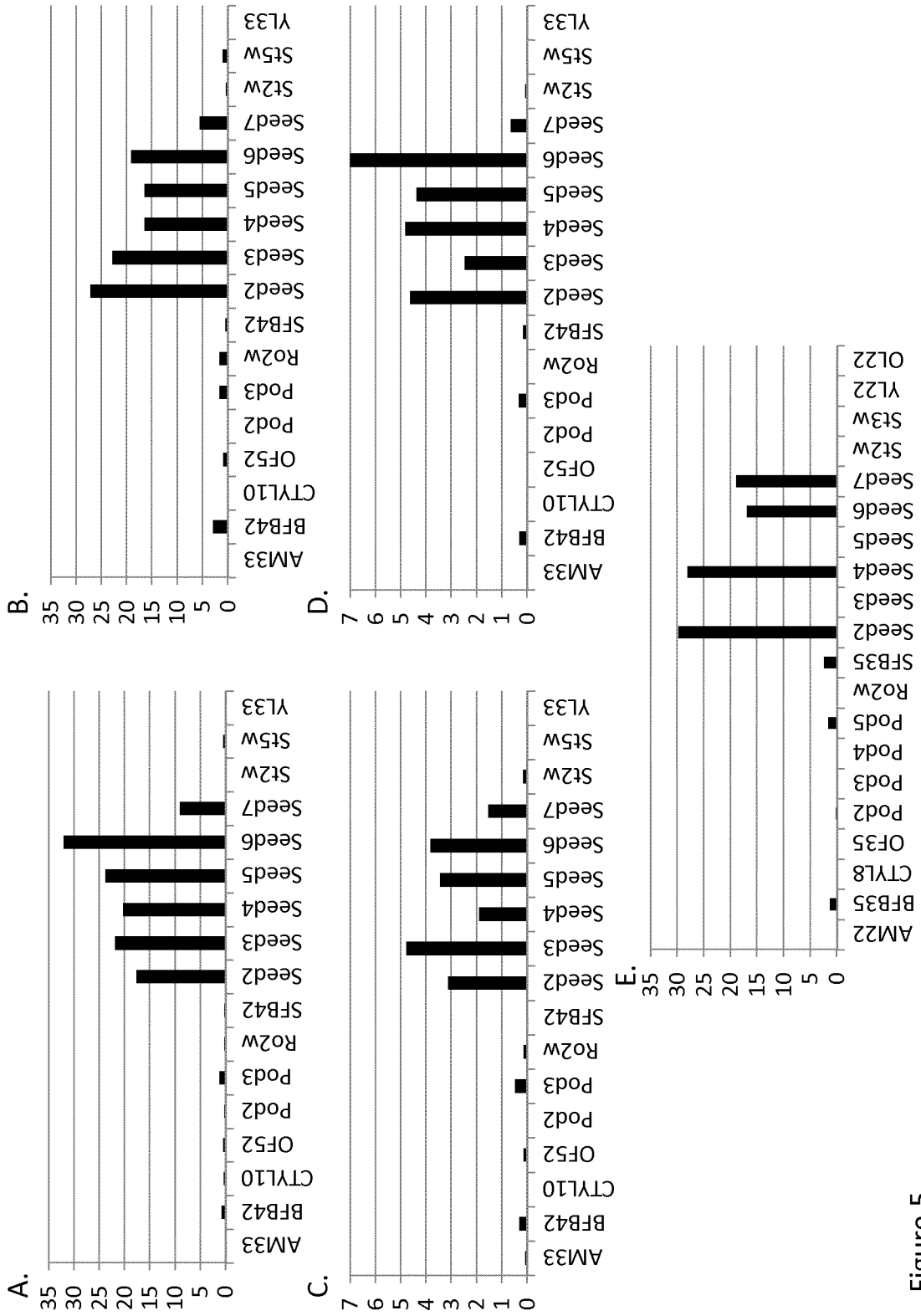


Figure 5

SEQ ID NO: 3 1042 gtgctagagtcctgtatgagactgtgatgtggcactctatccattggtggaagaagatttgctttgctgac
 SEQ ID NO: 4 998 tagttagagtcctgtatgagattgtgatgtggcgtctatccgttggggaagaatatttgctttgctgac
 SEQ ID NO: 5 993 gtgctagagtcctgtatgagactgtgatgtggcactctatccattggtggaagaagatttgctttgctgac
 SEQ ID NO: 6 989 tagttagagtcctgtatgagattgtgatgtggcgtctatccgttggggaagaatatttgctttgctgac
 SEQ ID NO: 7 979 gtgctagagtcctgtatgagattgtgatgtggcactctatccattggtggaagaagatttgctttgctgac

 SEQ ID NO: 3 1112 aactaacaagatttgggttatcacaacaaattggagtcgggtatataataacgctctagagaaggctcaa
 SEQ ID NO: 4 1068 aactaacaagatttgggttatcacaacaaattggagtcgggtatataataacgctctagagaaggctcaa
 SEQ ID NO: 5 1063 aactaacaagatttgggttatcacaacaaattggagtcgggtatataataacgctctagagaaggctcaa
 SEQ ID NO: 6 1059 aactaacaagattcgggttatcacaacaaattggagtcgggtatataataacgctctagagaaggctcaa
 SEQ ID NO: 7 1049 aactaacaagatttgggttatcacaacaaattggagtcgggtatataataacgctctagagaaggctcaa

 Promoter consensus sequence
 SEQ ID NO: 3 1182 gtatc**a**gtctaataactggttcaggatccgggttagagaaaaacccgggagagtgtgacgtgtggaagttagc
 SEQ ID NO: 4 1138 gtatc**a**gtctaataactggttcaggatccgggttagagaaaaacccgggagagtgtgacgtgtggaagttagc
 SEQ ID NO: 5 1133 gtatc**a**gtctaataactggttcaggatccgggttagagaaaaacccgggagagtgtgacgtgtggaagttagc
 SEQ ID NO: 6 1129 gtatc**a**gtctaataactggttcaggatccgggttagagaaaaacccgggagagtgtgacgtgtggaagttagc
 SEQ ID NO: 7 1119 gtatc**a**gtctaataactggttcaggatccgggttagagaaaaacccgggagagtgtgacgtgtggaagttagc

 UTR consensus sequence 1
 SEQ ID NO: 3 1252 gctctggattctttgagtcataagctcaaagtgacaaaaaacaatattttgtaac-----
 SEQ ID NO: 4 1208 actctggattctttgagtcataagctcaaagtgacaaaaaacaatattttgtaac-----
 SEQ ID NO: 5 1203 actctggattctttgagtcataagctcaaagtgacaaaaaacaatattttgtaac-----
 SEQ ID NO: 6 1199 actctggattctttgagtcataagctcaaagtgacaaaaaacaatattttgtaac-----
 SEQ ID NO: 7 1189 actctggattctttgagtcataagctcaaagtgacaaaaaacaatattttgtaac-----

Figure 6

SEQ ID NO: 3	1310	----gtatcaagaatttcctcttcgatatcttacttagagagagagagagagagatcttctcttcaa
SEQ ID NO: 4	1266	----gtatcaagaatttcctcttcgatatcttactt-----agagagagagagatcttctcttcaa
SEQ ID NO: 5	1261	----gtatcaagaatttcctcttcgatatcttacttagagagagagagagagatcttctcttcaa
SEQ ID NO: 6	1257	----gtatcaagaatttcctcttcgatatcttactta---agagagagagagatcttctgcttcaa
SEQ ID NO: 7	1259	taacgtatcaagaatttcctcttcgatatcttactt----agagagagagagatcttctcttcaa
		<u>UTR consensus sequence 2</u>
SEQ ID NO: 3	1376	gttctagagctttctccgccgatttcatctcctttagcc
SEQ ID NO: 4	1326	gttctagagctttctccgccgatttcatctcctttagcc
SEQ ID NO: 5	1327	gttctagagctttctccgccgatttcatctcctttagcc
SEQ ID NO: 6	1320	gttctagagctttctccgccgatttcatctcctttagcc
SEQ ID NO: 7	1325	gttctagagctttctccgccgatttcatctcctttagcc
		<u>UTR consensus sequence 3</u>

Figure 6 (continued)

SEQ ID NO: 3 1415 **atgg**taattaaaaaaaaa-----ctattttcagtccttttgccttcatatttcacgctctctctggatt
 SEQ ID NO: 4 1365 **atgg**taattaaaaaaaaa----ctgttttcagtcctttcttgccttcatatttcacgcttctctggatt
 SEQ ID NO: 5 1366 **atgg**taattaaaaaaaaa-----ctattttcagtcctttcttgccttcatatttcacgctctctctggatt
 SEQ ID NO: 6 1359 **atgg**taattaaaaaaaaa----ctattttcagtcctttcttgccttcatatttcacgcttctctggatt
 SEQ ID NO: 7 1364 **atgg**taattaaaaaaaaa----ctattttcagtcctttcttgccttcatatttcacgcttctctggatt
 Intron consensus sequence 1
 SEQ ID NO: 3 1481 ctctgctaattttggattgtggtatggccttttacgttctagggaaatcgatggaaacaaaaaagaaaaact
 SEQ ID NO: 4 1432 ctctgctaattttggattgtggtatggccttttacgttctagggaaatcgatggaaacaaaaaagaaaaact
 SEQ ID NO: 5 1430 ctctgctaattttggattgtggtatggccttttacgttctagggaaatcgatggaaacaaaaaagaaaaact
 SEQ ID NO: 6 1429 ctctgctaattttggattgtggtatggccttttacgttctagggaaatcgatggaaacaaaaaagaaaaact
 SEQ ID NO: 7 1430 ctctgctaattttggattgtggtatggccttttacgttctagggaaatcgatggaaacaaaaaagaaaaact
 Intron consensus sequence 2
 SEQ ID NO: 3 1551 gaaatgcatgctatatgttagattttgtaattgttataaaataaagaaatagcagtagttccaagta
 SEQ ID NO: 4 1502 gaaatgcatgcatatgttagattttgtaattgttataaaataaagaaatagcagtagttccaagta
 SEQ ID NO: 5 1500 gaaatgcatgctatatgttagattttgtaattgttataaaataaagaaatagcagtagttccaagta
 SEQ ID NO: 6 1499 gaaatgcatgcatatgttagattttgtaattgttataaaataaagaaatagcagtagttccaagta
 SEQ ID NO: 7 1500 taaatgcatgcatatgttagattttgtaattgttataaaataaagaaatagcagtagttccaagta
 Intron consensus sequence 2
 SEQ ID NO: 3 1621 agtggttaaaaccacaatagattttatgtaaaacttgcattgtaaaacttcaactttaagtttgttcatgat
 SEQ ID NO: 4 1572 agtggttaaaaccacaatagattttatgtaaaacttgcattgtaaaacttcaacttcaagtttgttcat---
 SEQ ID NO: 5 1570 agtggttaaaaccacaatagattttatgtaaaacttgcattgtaaaacttcaacttcaagtttgttcatgat
 SEQ ID NO: 6 1569 agtggttaaaaccacaatagattttatgtaaaacttgcattgtaaaacttcaacttcaagtttgttcat---
 SEQ ID NO: 7 1570 agtggttaaaaccacaatagattttatgtaaaacttgcattgtaaaacttcaacttcaagtttgttcatgat

Figure 7

```

SEQ ID NO: 3      1691 gatcttgatagcgtg-aaaatcaagaatcttagctttaaaagtcattggaggaggctgctagtgtaggttg
SEQ ID NO: 4      1639 tatcttgatagc-----
SEQ ID NO: 5      1640 gatcttgatagcgtg-aaaatcaagaatcttagctttaaaagtcattggaggaggctgctagtgtaggttg
SEQ ID NO: 6      1636 tatcttgatagc-----
SEQ ID NO: 7      1640 gatcttgatagcgtggaaaatcaagaatcttagctttaaaagtcattggaggaggctgctagtgtaggttg
                Intron consensus 3
    
```

Figure 7 (continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058388

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82
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)
C12N
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/122980 A2 (EVOGENE LTD [IL]; EMMANUEL EYAL [IL]; RONEN GIL [IL]; SAVIR NOA [IL]) 16 October 2008 (2008-10-16) page 71; sequences 662, 798 -----	1
X	DATABASE EMBL [Online] 9 October 2002 (2002-10-09), "oei86f11.b1 B.oleracea002 Brassica oleracea genomic, DNA sequence.", XP002770413, retrieved from EBI accession no. EM GSS:BH988060 Database accession no. BH988060 sequence ----- -/--	1

Further documents are listed in the continuation of Box C.

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 23 May 2017	Date of mailing of the international search report 13/06/2017
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Bilang, Jürg

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/058388

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/281570 A1 (ABBITT SHANE [US] ET AL) 4 November 2010 (2010-11-04) the whole document	1-23
A	----- TOMOHIRO UEMURA ET AL: "Systematic Analysis of SNARE Molecules in <i>Arabidopsis</i> : Dissection of the post-Golgi Network in Plant Cells", CELL STRUCTURE AND FUNCTION., vol. 29, no. 2, 1 January 2004 (2004-01-01), pages 49-65, XP055375122, JP ISSN: 0386-7196, DOI: 10.1247/csf.29.49 the whole document	1-23
A	----- SANG-JIN KIM ET AL: "News and Views into the SNARE Complexity in Arabidopsis", FRONTIERS IN PLANT SCIENCE, vol. 3, 1 January 2012 (2012-01-01), pages 28/1-28/6, XP055375124, DOI: 10.3389/fpls.2012.00028 the whole document -----	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2017/058388

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008122980	A2	16-10-2008	
		AU 2008236316	A1 16-10-2008
		BR PI0809796	A2 07-10-2014
		CA 2683143	A1 16-10-2008
		CN 101854798	A 06-10-2010
		EP 2154946	A2 24-02-2010
		MX 341624	B 26-08-2016
		US 2010154077	A1 17-06-2010
		US 2013291223	A1 31-10-2013
		US 2016348125	A1 01-12-2016
		WO 2008122980	A2 16-10-2008
		ZA 200907805	B 27-10-2010

US 2010281570	A1	04-11-2010	NONE
