

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 February 2007 (08.02.2007)

PCT

(10) International Publication Number
WO 2007/015238 A2

(51) International Patent Classification:

A01H 1/00 (2006.01)

(21) International Application Number:

PCT/IL2006/000887

(22) International Filing Date: 31 July 2006 (31.07.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/703,878 1 August 2005 (01.08.2005) US

(71) Applicant (for all designated States except US): **YEDA RESEARCH AND DEVELOPMENT CO. LTD.**

[IL/IL]; At The Weizmann Institute Of Science, P.o. Box 95, 76100 Rehovot (IL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **WEISSMAN, Sarit** [IL/IL]; 43b Nordau Street, 75263 Rishon-lezion (IL). **FELDMAN, Moshe** [IL/IL]; 48 HateEna Street, 99797 Carmei Yosef (IL). **GRESSEL, Jonathan** [IL/IL]; 15 HaYarden Street, 76604 Rehovot (IL).

(74) Agents: **G.E. EHRLICH (1995) LTD.** et al.; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF PREVENTING GENE FLOW IN PLANTS

(57) Abstract: Methods of reducing stable transgene introgression from cultivated transgenic plants to non-cultivated plants are provided. Specifically, the present invention provides methods of producing transgenic plants having an exogenous genomically integrated sequence that is incapable of stably introgressing into related crops or wild or weedy species.



WO 2007/015238 A2

METHODS OF PREVENTING GENE FLOW IN PLANTS

FIELD AND BACKGROUND OF THE INVENTION

5 The present invention relates to methods of reducing gene flow from cultivated transgenic plants to non-cultivated plants and more particularly, to methods of producing transgenic plants having an exogenous genomically integrated sequence which is incapable of introgressing into related crops or wild or weedy species.

10 One of the major concerns regarding the cultivation of transgenic crops is the uncontrolled spread of transgenes among fields to related wild species or to other varieties of the transgenic species. This is of particular importance when transgenic herbicide resistant genes are introduced. In 2004, approximately 58.6 million hectares of a total of >80 million hectares planted of transgenic crops were herbicide-resistant crops. Transgenes can flow from herbicide resistant crops to other varieties of the
15 same crop or to fully or partially compatible wild or weedy species through cross pollination. Thus, herbicide resistance in oilseed rape has moved from one variety to others in oilseed rape, and from cultivated rice (*Oryza sativa*) to weedy red rice (*Oryza sativa*). Other transgenes that may confer a selective advantage on a related weed or wild species can move at a similar rate in a similar manner.

20 Recently, a commercial release of new transgenic herbicide resistant wheat was abandoned. The reasons for this were not fully stated, but it was implied, in part, that the transgene would easily escape into populations of weedy relatives, notably *Aegilops* species. *Ae. cylindrica* is a pernicious weed in the US western grain belt (Jasieniuk, M. et al., 2001; Stone, A. E. & Peeper, T. F., 2004) although not in its
25 center of origin in northern Asia Minor. *Ae. longissima*, previously a species that grows on dry plains on non-agricultural rocky outcroppings near fields, is increasingly becoming a pesky weed in southern Israel. It is not clear whether that is due to introgression of wheat genes, evolution by itself, and/or the availability of an ecological niche due to the eradication of other grass weeds. Many other *Aegilops*
30 and wheat-related species reside in ruderal ecosystems in close proximity to wheat. Of these several *Aegilops* species, namely, *Ae. cylindrica* ($2n = 4X = 28$, genomes CCDD), *Ae. ventricosa* ($2n = 4x = 28$; genome DDUⁿUⁿ), *Ae. crassa* tetraploid ($2n = 4x = 28$; genome DDM^{cr}M^{cr}) and hexaploid ($2n = 6x = 42$; genome DDD²D²M^{cr}M^{cr}), *Ae. vavilovii* ($2n = 6x = 42$; genome DDM^{cr}M^{cr}S^lS^l), *Ae. juvenalis* ($2n = 6x = 42$;
35 genome DDM^{cr}M^{cr}UU) and *Ae. tauschii* ($2n = 2x = 14$; genome DD) (Kihara, H.

1954; Kimber, G. & Tsunewaki, K., 1988), has one of their genomes homologous to the D genome of bread wheat *Triticum aestivum* ($2n = 6X = 42$, genomes BBAADD). Thus, it was suggested that only wheat with transgenes on the A or B genome be released, to prevent gene flow to these species (Gressel, J., 2002; Wang, Z. N., et al., 5 2001). This is specious, as breeders have commonly incorporated alien genes from homoeologous chromosomes of *Aegilops* species into wheat (Dhaliwal, H. S., et al., 2002; Ganeva, G et al., 2000; Martin-Sanchez, J. A. et al. 2003; Spetsov, P., et al., 1997; Sharma, H. et al. 1995).

Studies have demonstrated gene flow from wheat to related genera. Groups in 10 Oregon and Switzerland have demonstrated that hybrids easily form between wheat and *Ae. cylindrica*, with wheat genes remaining in the first two backcrosses, but have not yet demonstrated that wheat gene introgression is stabilized in the C genome of *Ae. cylindrica*, which is homoeologous to the genomes of bread wheat (Wang, Z. N., Z., et al., 2001; Guadagnuolo, R., et al., 2001a; Hegde, S. G. & Waines, J. G., 2004; 15 Morrison, L. A., et al., 2002a, b; Wang, Z. N. et al., 2000; Wang, Z. N. et al., 2002; Zemetra, R. S., et al., 1998). In addition, the present inventors have shown that a specific wheat DNA sequence appears sporadically in *Ae. peregrina* (*Ae. variabilis*) ($2n = 4X = 28$, genomes S^VS^VUU) in nature (Weissmann, S., et al. 2005), and have also demonstrated that wheat genes can introgress and are stable in *Ae. peregrina* 20 BC₃F₂ plants with 28 chromosomes after manual hybrid formation between *Ae. peregrina* and bread wheat and backcrossing to *Ae. peregrina* (Weissmann, Feldman and Gressel, unpublished data). Similarly, wheat-specific DNA sequences have been found in an accession of sea barley (*Hordeum marinum*), which had the appearance of the weed (Guadagnuolo, R., et al., 2001b). It is not clear that the DNA has 25 introgressed into a *Hordeum* chromosome as no chromosome counts nor progeny segregation analyses were reported.

Farmers need transgenic wheat varieties to deal with problems where breeding cannot help, or where breeding is slow. Weed control is one problem where breeding has been of little assistance (Lyon, D. J., et al., 2002). Graminicides (herbicides that 30 control grass weeds) are continually falling by the wayside due to grass weeds evolving resistance, either by mutating the herbicide target sites to non binding forms, or up-regulating cytochrome P450s that degrade the herbicides, the same mode used by wheat to be resistant to graminicides (Gressel, J., 2002; Gressel, J., 1988). Much of Australian wheat lands as well as smaller areas scattered throughout the world are

plagued by the weed *Lolium rigidum* (annual or rigid ryegrass) (De Prado, J. et al., 2005; Neve, P., et al., 2004; Yu, Q., et al., 2004), which has evolved resistance to all selective graminicides commonly used in wheat. *Alopecurus myosuroides* (blackgrass) has evolved the same way in Britain, but has been spread to a less extent
5 (Price, L., et al., 2003; Cocker, K. M., et al., 1999). In North America there are large areas without a graminicide to control *Avena* spp. (wild oats) (Beckie, H. J. et al., 1999; Mengistu, L. W., et al., 2003; Kern, A. J. et al., 2002), in other areas there are limited graminicide choices to deal with *Setaria* (foxtail) species (Delye, C., et al., 2004; De Prado, R., et al., 2004; Volenberg, D. S., et al., 2002; De Prado, R., et al.,
10 2000). The extent of these resistance problems is continuously documented on the web site <http://www.weedscience.org>.

It is clear that resistant grass weeds will rear their ugly heads throughout the wheat lands of the world. The chemical industry has been of little help; it is decades since a graminicide for wheat with a new mode of action has been released, or a pre-
15 release described at scientific meetings. If selectivity of new herbicides for wheat is based on the same P450s used by wheat at present, then some grass weeds will be resistant to new graminicides before they are tested (Gressel, J. 2002; Gressel, J. 1988; Preston, C., 2004). Thus, transgenes, where genes encode novel mechanisms of selectivity not found in any plants, offer a solution although no solution is forever in
20 agriculture (Yenish, J. R. & Young, F. L., 2004). This is especially true with the weedy relatives of wheat, such as *Ae. cylindrica*, which cannot be controlled by wheat selective herbicides, but could be controlled by transgenic herbicide resistance, or wheat with specific mutations conferring herbicide resistance (Ball, D. A., et al., 1999). Unfortunately, the herbicide resistant wheat marketed has the mutant gene
25 localized on the D genome of wheat (Anderson, J. A., et al., 2004), which homologously recombines with D genome of *Ae. cylindrica*. Indeed, a high rate of wheat D genome retention was found in manually produced BC₂ plants of wheat X *Ae. cylindrica* hybrids backcrossed to *Ae. cylindrica* (Kroiss, L. J. et al., 2004).

Similarly, a new violent race of stem rust has appeared in Africa on wheat
30 lines having the commonly used rye *Sr31* gene, for which no resistance had previously been reported anywhere in the world (<http://www.cdl.umn.edu/crb/1999crb/99crb2.html>). Some sort of transgenic rust resistance may be a quicker fix than trying to find resistance genes within the diversity of wheat. One does not want transgenic disease resistance stably

introgressing into wild relatives, which would render them healthier and more competitive with crops. If wheat were transformed to greater mineral-nutrient use efficiency, the transgenes would be of great utility to its wild/weedy relatives, to the detriment of agriculture.

5 Other allopolyploid crops such as cotton and oilseed rape also have needs that transgenic technologies have fulfilled, as is apparent from farmer acceptance. As discussed below, oilseed rape can easily transfer genes to one of its weedy/wild diploid progenitors.

As transgenics are imperative to the future of wheat (Gressel, J., 2002;
10 Gressel, J., 1988) and other allopolyploid crops, the issue of gene flow to related weeds and wild species must be dealt with (Ellstrand, N. C. 2003; Stewart, C. N., et al., 2003; Snyder, J. R., 2000). Thus, herbicide or disease resistant, nutrient efficient weedy or ruderal species of the Triticeae can spread in wheat fields, when all other grass weeds are eliminated, or diseased, creating a niche for these species that had
15 been less competitive than the other grass weeds.

As mentioned before, inserting transgenes to allopolyploid crop chromosomes that are homoeologous to related weed/wild species does not preclude transfer. Other solutions have been suggested such as GURT (Genetic Use Restriction Technologies) (terminator) but they have not yet been demonstrated to work in the field (Oliver, M.
20 J., et al., 2004; U.S. Pat. 5,723,765, 1998 to Oliver, M. J., et al.). This system allows crosses between the wild/weedy relatives or other varieties in the areas used for seed production, so it is also "leaky".

It has also been proposed that transforming transgenes into the chloroplast genome (plastome) would prevent gene flow due to the maternal mode of plastid
25 inheritance (Daniell, H., 2002; Maliga, P., 2004). This is also a highly leaky technology with 0.4 % pollen transmission of plastid genes in the field between the crop *Setaria italica* (Italian millet) and its biologically con-specific (i.e., same species), weedy progenitor *S. viridis* (Wang, T. et al., 2004) as well as in other cases (Darmency, H. 1994). Moreover, in many natural wheat x wild relative hybrids,
30 wheat was the predominant male parent (Morrison, L. A., 2002b), but the wild species can pollinate wheat producing the same F₁ plants albeit bearing the wheat plastome.

As gene containment systems seem leaky, it was suggested to augment them with transgenic mitigation, i.e. coupling the gene of choice in tandem with a gene that is neutral or useful to the crop, but would render weedy offspring unfit to compete

(Gressel, J. 1999, US Pat. Appl. No. 20040172678 to Gressel and Al-Ahmad). Such mitigating genes, which would be inherited as a genetically linked group, include dwarfing (increases crop yield) and anti-shattering (preventing the premature seed drop that keeps weed seeds from being harvested and removed from the field). The utility of mitigation has been demonstrated through the stage of transgenic screen house in two species (Al-Ahmad, H., et al., 2004; Al-Ahmad, H., et al., 2005a and b). It is a technology that should be coupled with other technologies to further lower the risk of transgene flow.

Polyploid crops can be divided into two groups, those that arose from a single progenitor by genome duplication, such as potato, alfalfa, strawberry, and yam, and those 'allopolyploids' such as durum and bread wheat, oilseed rape, cotton, sugarcane, and tobacco, that resulted from the hybridization of more than one progenitor of different, but closely related species having homoeologous chromosomes. Despite the synteny and similarity between the constituent genomes, pairing of chromosomes during meiosis of allopolyploid crops appears similar to that of diploid crops, namely, only bivalent pairing.

There is thus a widely recognized need for, and it would be highly advantageous to have, a method of preventing genetic introgression of plant transgenes into wild type plants devoid of the above limitations.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, the genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant thereby preventing the stable transgene introgression into the related, non-cultivated plant.

According to another aspect of the present invention there is provided a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a genetic locus of the transgenic cultivated

plant, the genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant.

According to yet another aspect of the present invention there is provided a method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, the genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant thereby preventing the transgene flow into the related, non-cultivated plant.

According to still another aspect of the present invention there is provided a method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a centromeric region of a chromosome of the transgenic cultivated plant thereby preventing the transgene flow into the related, non-cultivated plant.

According to an additional aspect of the present invention there is provided a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a centromeric region of a chromosome of the transgenic cultivated plant.

According to yet an additional aspect of the present invention there is provided a method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a centromeric region of a chromosome of the transgenic cultivated plant.

According to still an additional aspect of the present invention there is provided a method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, the genetic locus being in linkage disequilibrium with a locus including a gene capable of preventing homoeologous recombination, thereby preventing the stable introgression into the related, non-cultivated plant.

According to a further aspect of the present invention there is provided a transgenic cultivated plant characterized by reduced stable transgene introgression to

a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a genetic locus of the transgenic cultivated plant, the genetic locus being in linkage disequilibrium with a locus including a gene capable of preventing homoeologous recombination.

5 According to yet a further aspect of the present invention there is provided a method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, the genetic locus being in linkage disequilibrium with a locus including a gene
10 capable of preventing homoeologous recombination thereby reducing introgression of the exogenous polynucleotide into the related, non-cultivated plant.

 According to further features in preferred embodiments of the invention described below, the exogenous polynucleotide comprises a nucleic acid sequence encoding a polynucleotide or a polypeptide product.

15 According to still further features in the described preferred embodiments the genetic locus being in linkage disequilibrium with the *Ph1* locus is set forth by SEQ ID NO:13, 14, 15, 16, 17 and 39 .

 According to still further features in the described preferred embodiments the transgenic cultivated plant is wheat.

20 According to still further features in the described preferred embodiments the wheat is *Triticum aestivum* ssp. *aestivum* or *Triticum turgidum* ssp. *durum*.

 According to still further features in the described preferred embodiments the related, non-cultivated plant is selected from the group consisting of *Aegilops* spp., *Hordeum* spp., *Elymus* spp., *Agropyron* spp., *Eremopyrum* spp., *Secale* spp.,
25 *Dasyphyrum* spp., *Heteranthelium* spp., *Amblyopyrum* spp., *Henrardia* spp., *Thinopyrum* spp., *Leymus* spp., *Psathyrostachys* spp., *Hystrix* spp., *Hordelymus* spp., *Taeniatherum* spp., and *Crithopsis* spp.

 According to still further features in the described preferred embodiments the exogenous polynucleotide comprises a nucleic acid sequence encoding a suppressor
30 of a gene or gene product lethal to the related, non-cultivated plant.

 According to still further features in the described preferred embodiments the method further comprising inserting a second exogenous polynucleotide into a second genetic locus of the transgenic cultivated plant, the second genetic locus being in random association with the *Ph1* locus of the transgenic cultivated plant.

According to still further features in the described preferred embodiments the transgenic cultivated plant further comprising a second exogenous polynucleotide positioned in a second genetic locus of the transgenic cultivated plant, the second genetic locus being in random association with the *Ph1* locus of the transgenic cultivated plant.

According to still further features in the described preferred embodiments the second exogenous polynucleotide comprises a nucleic acid sequence encoding a product lethal to the related, non-cultivated plant.

According to still further features in the described preferred embodiments the second exogenous polynucleotide further comprises a second nucleic acid sequence encoding a transgene.

According to still further features in the described preferred embodiments the transgene is a polynucleotide or a polypeptide.

According to still further features in the described preferred embodiments the polynucleotide or polypeptide product endows the transgenic cultivated plant with a commercially desirable trait selected from the group consisting of herbicide resistance, disease resistance, insect resistance and nematode resistance, environmental stress resistance, high productivity, modified agronomic quality, enhanced yield, modified ripening, and bioremediation.

According to still further features in the described preferred embodiments the exogenous polynucleotide further comprises a promoter for directing an expression of the nucleic acid sequence in the transgenic cultivated crop.

According to still further features in the described preferred embodiments the promoter is selected from the group consisting of FMV, 35S, E8, E4, E17, J49, 2A11, and Tap1.

According to still further features in the described preferred embodiments the product lethal to the related, non-cultivated plant is barnase.

According to still further features in the described preferred embodiments the suppressor of the product lethal to the related, non-cultivated plant is *barstar*.

According to still further features in the described preferred embodiments the centromeric region of the transgenic cultivated plant is a sub-centromeric region of the transgenic cultivated plant.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a method of preventing stable gene introgression into non-cultivated plants.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 is an AFLP analysis depicting introgression of DNA from the B genome of *T. aestivum* (line TAA01) into *Ae. peregrina* (line TKE02). The introgressed band (marked by an arrow) is present in *T. aestivum* [TAA01, genome BBAADD], *T. turgidum* ssp. *durum* (TTR (lines TTR298 and TTR16), genome BBAA), F_1 of the cross between *T. aestivum* and *Ae. peregrina* (F_1 , $2n=35$; genome S^vUBAD), BC_3F_2 [having $2n=28$ chromosomes (14 bivalents at meiotic metaphase)], and BC_3 [having 29 chromosomes (14 bivalents + 1 univalent, presumably of *T. aestivum*)], but missing from *Ae. peregrina* (TKE02 genome S^vS^vUU), *T. urartu* [TMU (lines TMU38 and TMU05), genome AA), and *Ae. tauschii* [TQ (lines TQ96, TQ81 and TQ71), genome DD].

FIG. 2 is an AFLP analysis depicting introgression from the D (band No. 1) and A (band No. 2) genomes of *T. aestivum* (TAA01) into *Ae. peregrina* (TKE02). Band No. 1 is present in *T. aestivum* [TAA01 (genome BBAADD)], *Ae. tauschii* (TQ71, TQ81 and TQ96); genome DD), F₁ of the cross between *T. aestivum* and *Ae. peregrina* [having 2n = 28 chromosomes (14 bivalents at meiotic metaphase)], and BC₃ [having 29 chromosomes (14 bivalents + 1 univalent, presumably of *T. aestivum*), but missing from *Ae. peregrina* [TKE02 genome S^vS^vUU], *T. turgidum* ssp. *durum* [(TTR16 and TTR298); genome BBAA], and *T. urartu* [(TMU05 and TMU38); genome AA). Band No. 2 is present in *T. aestivum* [TAA01 (genome BBAADD)], *T. turgidum* ssp. *durum* [(TTR16 and TTR298); genome BBAA], *T. urartu* [(TMU05 and TMU38); genome AA], F₁ of the cross between *T. aestivum* and *Ae. peregrina* (F₁, 2n 35; genome S^vU BAD), BC₃F₂ [having 2n = 28 chromosomes (14 bivalents at meiotic metaphase)], and BC₃ [having 29 chromosomes (14 bivalents + 1 univalent, presumably of *T. aestivum*), but missing from *Ae. tauschii* [(TQ96, TQ81 and TQ71), genome DD] and *Ae. peregrina* [TKE02 genome S^vS^vUU].

FIG. 3 is an AFLP analysis depicting introgression of a band from an undetermined genome of *T. aestivum* (TAA01) into *Ae. peregrina* (TKE02). The introgressed band (marked by an arrow) is present in *T. aestivum* (TAA01, genome BBAADD), *T. turgidum* ssp. *durum* (TTR298 and TTR16; genome BBAA), F₁ of the cross between *T. aestivum* and *Ae. peregrina* [F₁, 2n = 35; genome S^vUBAD], BC₃F₂ [having 2n=28 chromosomes (14 bivalents at meiotic metaphase)], and BC₃ [having 29 chromosomes (14 bivalents + 1 univalent, presumably of *T. aestivum*),, *T. urartu* (TMU05 and TMU38; genome AA), and *Ae. tauschii* (TQ96, TQ81 and TQ71; genome DD), but missing from *Ae. peregrina* [TKE02, genome S^vS^vUU]. Note that this band segregates; it is missing from one of the BC₃ plants (right BC3 lane) and from four BC₃F₂ plants.

FIG. 4 is a schematic illustration depicting random segregation of chromosomes in the F₁ hybrids (due to the presence of *Ph1* gene that suppresses homoeologous pairing) may lead to the formation of *Ph1* lacking plants in backcross generations. The absence of *Ph1* causes an increase of homoeologous pairing and thus promotes recombination and introgression in backcross plants.

FIG. 5 is a schematic illustration depicting two failsafe mechanisms (b and c) to suppress the movement of transgene from bread wheat into a wild relative and one failsafe mechanism (a) which is used by other studies. a, possible transgene

movement, by homoeologous recombination, from wheat chromosomes into wild relatives without linkage to *Ph1*; b, transgene movement by homoeologous recombination is suppressed by linkage of the transgene to *Ph1* on the long arm of wheat chromosome 5B; c, a double failsafe mechanism that includes the placement of a transgene in a tandem construct with *barnase*, a gene encoding a lethal RNase, on any chromosome arm other than 5BL, and by inserting *barstar* on chromosome arm 5BL in proximity to *Ph1*. *Barstar* encodes a protein that suppresses the specific RNase produced by *barnase*. Any backcross progeny having the transgene without chromosome arm 5BL will die. The presence of *Ph1*, in backcross plants containing 5BL, will prevent the establishment of *barstar* and the transgene (if present) in the wild population by preventing homoeologous pairing.

FIG. 6 is a photomicrograph depicting chromosomal staining of an F₁ hybrid plant. The chromosomes were stained with acetocarmine. Note the expected number of 35 chromosomes (21 bread wheat chromosomes + 14 *Ae. peregrina* chromosomes) in the F₁ hybrid plant of *Ae. peregrina* x *T. aestivum*.

FIGs. 7a-d are photomicrographs depicting chromosomal staining of BC₃F₂ plants derived from backcrossing the F₁ hybrid *Ae. peregrina* x *T. aestivum* three times to *Ae. peregrina*. Figure 7a – a stabilized BC₃F₂ plant containing 28 chromosomes. This plant is among the 15 BC₃F₂ plants that were used to analyze introgression from *T. aestivum* into *Ae. peregrina*. Figure 7b - BC₃ plants contained trivalents (marked by an arrow) at meiosis. This may indicate a possible translocation from *T. aestivum* into *Ae. peregrina*. One (or two) of the homologous chromosomes of *Ae. peregrina* may contain a wheat segment homologous to that of the third chromosome (possibly a wheat chromosome) attached to the bivalent. Figure 7c - In meiosis, all BC₃F₂ plants with 28 chromosomes had 14 bivalents. Figure 7d – A meiotic cell of wild type parental species *Ae. Peregrina*. Note that the BC₃F₂ plants shown in Figure 7c exhibit a significantly ($P = 1.07 \times e^{-46}$) higher number of rod bivalents (range 3-7, average 4.03) as compared to their wild type parental species *Ae. peregrina* (range 1-4, average 2.01) shown in Figure 7d.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of transgenic plants and methods of preventing stable introgression of transgenes into non-target plants. The present method can be utilized to generate a transgenic cultivated plant which is failsafe, *i.e.*, is unable to introgress genes into a genome of related, non-cultivated plants such as weeds.

The principles and operation of the method of preventing stable genome introgression according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Domesticated wheat (durum and bread wheat) are in dire need of transgenic traits such as herbicide and disease resistance, especially in light of the recent evolution of herbicide resistant grass weeds and an intractable new strain of rust. However, such transgenes can be transferred to wild/weedy relatives of the wheat through cross-pollination. Such naturally occurring gene flow has been documented in closely related *Aegilops* spp. (Weissmann et al., 2005) as well as in a more distantly related barley (*Hordeum*) spp (Guadagnuolo, R., et al., 2001a). Other allopolyploid crops, such as oilseed rape and cotton have similar biotic and abiotic constraints that genetic engineering can rapidly solve. This is evident from the large number of experimental as well as commercial releases of transgenic oilseed rape and cotton from the Animal and Plant Health Inspection Services website (APHIS; <http://www.aphis.usda.gov/>).

Studies have focused on the formation of crop-wild inter-generic hybrids, and assumed that introgression between homoeologous genomes is mainly the result of homoeologous pairing in the hybrid (Guadagnuolo et al. 2001a; Hegde and Waines 2004; Seefeldt et al. 1998; Snyder et al. 2000; Wang et al. 2001; Zemetra et al. 1998). However, due to the *Ph1* gene, which suppresses pairing between homoeologous chromosomes (Wall et al. 1971, Genetical Research 18: 329-339), F₁ hybrid plants between wild relatives and durum or bread wheat are characterized by low homoeologous pairing [Jauhar, P.P., Peterson, T.S. (2001). Hybrids between durum

wheat and *Thinopyrum junceiforme*: Prospects for breeding for scab resistance. *Euphytica* 118 (2): 127-136; Anamthawat-Jonsson and Bodvarsdottir, 1998, *Chromosome Research* 6: 339-343].

In sharp contrast to these studies, the present inventors have uncovered that the recombination events occurring between homoeologous chromosomes of cultivated wheat (*T. aestivum* cv. Chinese Spring; TAA01) and *Ae. peregrina* is significantly higher than the number of bivalents per cell observed in F1 hybrids between *T. aestivum* cv. Chinese Spring and *Ae. peregrina* TKE02 (Ozkan and Feldman 2001, *Genome* 44: 1000-1006.; Sears 1977, *Canadian Journal of Genetics and Cytology* 19: 585-593). As is shown in Figures 1-3 and is described in Example 1 of the Examples section that follows, the present inventors have uncovered 17 DNA fragments which introgressed from cultivated wheat (TAA01) to BC₃ and BC₃F₂ backcrosses. These results demonstrate that stable genomic introgression occurs in a frequency higher than expected during backcrosses of the transgenic plant to the wild type.

These results have led the present inventors to postulate that cultivated transgenic plants can be generated such that gene flow of the transgene to related wild/weedy relatives of the cultivated plant is substantially prevented. As is shown in Examples 2 and 3 of the Examples section that follows, the present inventors have uncovered methods of generating transgenic plants characterized by reduced stable transgene introgression.

Thus, according to one aspect of the present invention there is provided a method of generating a transgenic cultivated plant characterized by a reduced stable transgene introgression to a related, non-cultivated species.

As used herein the phrase "reduced stable transgene introgression" refers to a plant in which any transgene (e.g., an exogenous polynucleotide) being inserted into a genome thereof is characterized by reduced gene introgression. The phrase "gene introgression" or "transgene introgression", which is interchangeably used herein, refers to a stable recombination (transfer of nucleic acid sequences) between plant species having homoeologous chromosomes. Homoeologous chromosomes are non-identical yet related chromosomes of two related species from the same genus or related genera that have a common ancestor, but have diverged from each other during evolution. Such chromosomes are partial homologous, similar in synteny and in many sequences and thus can pair at meiosis. Thus, homoeologous chromosomes

share some homologous DNA sequences along with some significantly non-homologous DNA sequences.

The method according to this aspect of the present invention is effected by inserting an exogenous polynucleotide into a genetic locus that is in linkage disequilibrium with the *Ph1* locus of the transgenic cultivated plant.

The term "*Ph1* locus" as used herein, refers to the genomic location of a polynucleotide or a gene encoding the *Ph1* gene product on a chromosome region. In durum and bread wheat *Ph1* is located in the central region of the long arm of chromosome 5B (5BL) (Jampates, R. & Dvorak, J. 1986), about 1 centi-Morgan (cM) from the centromere (Sears, E. R., 1984). *Ph1* (or a *Ph1* homolog) exists only in polyploid species of *Triticum* having the B genome.

The phrase "linkage disequilibrium (LD)" refers to the non-random association between DNA markers or genes at two adjacent loci. LD is quantified with either Lewontin's parameter of association (D') or with Pearson correlation coefficient (r) [Devlin B, Risch N. (1995). A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics*. 29: 311-322.]. Two loci with a LD value of 1 are considered to be in complete LD. At the other extreme, two loci with a LD value of 0 are termed to be in linkage equilibrium. LD values according to the present invention for neighboring loci are selected above 0.1, preferably, above 0.2, more preferable above 0.5, more preferably, above 0.6, still more preferably, above 0.7, preferably, above 0.8, more preferably above 0.9, ideally about 1.0 to 1.0.

It will be appreciated that the genetic distance of two loci which are in linkage disequilibrium is smaller than the genetic distance (measured in centiMorgans) of loci which are in linkage equilibrium. Preferably, the genetic distance of the two loci of the present invention (*i.e.*, the locus in which the exogenous polynucleotide is inserted and the *Ph1* gene locus) is smaller than 50 centiMorgan, preferably smaller than 40 centiMorgan, more preferably smaller than 30 centiMorgan, more preferably smaller than 20 centiMorgan, more preferably smaller than 10 centiMorgan, more preferably smaller than 5 centiMorgan, more preferably smaller than 1 centiMorgan, most preferably in the range of 0 to 1 centiMorgan, wherein 0 centiMorgan refers to juxtaposed sequences.

Although the gene coding to *Ph1* has not yet been isolated, various sequences are genetically mapped to the *Ph1* locus using genetic studies (*e.g.*, recombination analyses between markers and the like). Recently, Griffiths S., et al., have localized

the *Ph1* locus to a 2.5-megabase interstitial region of wheat chromosome 5B (Nature Letters, 2006, 439: 749-752, incorporated herein by reference). This 2.5-megabase region was sequenced and a group of three *cdc2*-related genes was identified. Although the *cdc2* genes are good candidates for being involved in *Ph1* function, as *cdc2*-related genes affect chromosome condensation (<http://www.ihop-net.org/UniPub/iHOP/>), none of the genes that were identified in the region showed differences in expression between the presence and absence of *Ph1*.

Non-limiting examples of sequences (genes, markers or loci) which are in linkage disequilibrium with the *Ph1* locus in wheat, and which can be used according to this aspect of the present invention, are provided in Table 1, hereinbelow.

Table 1
Genes, markers and loci in linkage disequilibrium with the *Ph1* locus on the long arm of wheat chromosome 5B.

<i>SEQ ID No:</i>	<i>Name</i>	<i>coding</i>	<i>Distance from the centromere (break point interval)</i>	<i>GenBank accession No.</i>
39	BARC58	No	C-5BL6-0.29	BV211574
13	BARC74	No	5BL1-0.55-0.75	BV211851
14	BARC243	No	5BL16-0.79-1.00	BV211548
15	BARC1061	No	5BL9-0.76-0.79	BV211398
16	BARC88	No	5BL1-0.55-0.75	BV211800
17	BARC89	No	C-5BL6-0.29	BV211400

Table 1: Data from: <http://wheat.pw.usda.gov/GG2/index.shtml>, <http://ncbi.nlm.nih.gov/>. The *Ph1* locus is mapped to chromosomal interval 5L-0.53-0.53 (<http://wheat.pw.usda.gov/>, Wheat, Physical - Ta-Physical-Gill-5ABD).

According to one preferred embodiment of this aspect of the present invention, the genetic locus being in linkage disequilibrium with the *Ph1* locus is set forth by SEQ ID NO: 13, 16 or 17.

It will be appreciated that other loci of genes that are capable of preventing recombination between homoeologous chromosomes can be also used along with the present invention.

As used herein the phrase "transgenic cultivated plant" refers to a cultivated plant genetically modified to include an exogenous polynucleotide (*i.e.*, a transgene) in a genome thereof. The transgenic cultivated plant of the present invention can include the *Ph1* locus (e.g., wheat) or another locus of another gene capable of preventing homoeologous recombination. Preferably, the transgenic cultivated plant of the present invention is wheat. It will be appreciated that since the wheat genome

comprises three sets of complete genomes, *i.e.*, genomes A, B and D, the exogenous polynucleotide can be integrated into any one of the chromosomes of each genome (*i.e.*, chromosomes 1-7 of genome A, B or D).

Preferably, the wheat used by the present invention is *Triticum aestivum* ssp. *aestivum* and/or *Triticum turgidum* ssp. *durum*.

As used herein in the specification and in the claims section that follows, the phrase "related, non-cultivated plant" refers to any wild type species or related species or related genera of the cultivated plant including weeds. Non-limiting examples of related, non-cultivated plants that can introgress genes into wheat or vice versa include *Aegilops* spp., *Hordeum* spp., *Elymus* spp., *Agropyron* spp., *Eremopyrum* spp., *Secale* spp., *Dasypyrum* spp., *Heteranthelium* spp., *Amblyopyrum* spp., *Henrardia* spp., *Thinopyrum* spp., *Leymus* spp., *Psathyrostachys* spp., *Hystrix* spp., *Hordelymus* spp., *Taeniatherum* spp., *Crithopsis* spp and other species of the tribe Triticeae.

The term "weed" includes undesirable plants growing wild, especially those growing on cultivated ground to the disadvantage of a cultivated crop, lawn, or flower bed. In the broadest sense, the term weed is defined as all plants that grow in locations where they are undesired, including, for example: dicotyledonous weeds of the genera: *Abutilon*, *Amaranthus*, *Ambrosia*, *Anoda*, *Anthemis*, *Aphanes*, *Atriplex*, *Bellis*, *Bidens*, *Capsella*, *Carduus*, *Cassia*, *Centaurea*, *Chenopodium*, *Cirsium*, *Convolvulus*, *Datura*, *Desmodium*, *Emex*, *Erysimum*, *Euphorbia*, *Galeopsis*, *Galinsoga*, *Galium*, *Hibiscus*, *Ipomoea*, *Kochia*, *Lamium*, *Lepidium*, *Lindernia*, *Matricaria*, *Mentha*, *Mercurialis*, *Mullugo*, *Myosotis*, *Papaver*, *Pharbitis*, *Plantago*, *Polygonum*, *Portulaca*, *Ranunculus*, *Raphanus*, *Rorippa*, *Rotala*, *Rumex*, *Salsola*, *Senecio*, *Sesbania*, *Sida*, *Sinapis*, *Solanum*, *Sonchus*, *Sphenoclea*, *Stellaria*, *Taraxacum*, *Thlaspi*, *Trifolium*, *Urtica*, *Veronica*, *Viola*, *Xanthium*, weedy varieties of dicotyledonous crops of the genera: *Arachis*, *Beta*, *Brassica*, *Cucumis*, *Cucurbita*, *Helianthus*, *Daucus*, *Glycine*, *Gossypium*, *Ipomoea*, *Lactuca*, *Linum*, *Lycopersicon*, *Nicotiana*, *Phaseolus*, *Pisum*, *Solanum*, *Vicia*; monocotyledonous weeds of the genera: *Aegilops*, *Agropyron*, *Agrostis*, *Alopecurus*, *Apera*, *Avena*, *Brachiaria*, *Bromus*, *Cenchrus*, *Commelina*, *Cynodon*, *Cyperus*, *Dactyloctenium*, *Digitaria*, *Echinochloa*, *Eleocharis*, *Eleusine*, *Eragrostis*, *Eriochloa*, *Festuca*, *Fimbristylis*, *Heteranthera*, *Imperata*, *Ischaemum*, *Leptochloa*, *Lolium*, *Monochoria*, *Panicum*, *Paspalum*, *Phalaris*, *Phleum*, *Poa*, *Rottboellia*, *Sagittaria*, *Scirpus*, *Setaria*, *Sorghum*, *Thinopyrum* spp., *Leymus* spp., and *Psathyrostachys* and weedy varieties of

monocotyledonous crops of the genera: *Allium*, *Ananas*, *Asparagus*, *Avena*, *Hordeum*, *Oryza*, *Panicum*, *Saccharum*, *Secale*, *Sorghum*, *Triticale*, *Triticum*, *Zea*. The term further includes various forms of the crop species that are undesirable to agriculture: feral forms that have escaped cultivation and have evolved weedy characters, wild (uncultivated) forms that have escaped cultivation and evolved weedy characters undesirable, uncultivated interbreeding species related to the cultivated crop, other varieties of the crop that do not possess the same transgenes, and the transgenic crop when it is a volunteer weed in following crops.

According to one preferred embodiment of this aspect of the present invention, the transgenic cultivated plants are generated such that they harbor a gene which is beneficial to plant such as a commercially desirable trait. Non-limiting examples of commercially desirable traits include herbicide resistance, disease resistance, insect resistance, nematode resistance, environmental stress resistance (e.g., cold resistance and drought resistance), high productivity, modified agronomic quality, enhanced yield, modified ripening, and bioremediation. Table 2, herein below, summarizes the various genetic elements endowing desired traits into transgenic plants.

Table 2
Commercially desirable traits that have been engineered into crop plants

<i>Trait</i>	<i>Genetic Element</i>	<i>Source</i>
Fatty acid composition	delta(12)-fatty acid dehydrogenase (fad2),	<i>Glycine max</i>
Fatty acid composition	fatty acid desaturase (chemical mutagenesis)	NULL
Fatty acid composition	thioesterase (TE)	<i>Umbellularia californica</i>
Fertility restoration	barnase ribonuclease inhibitor barnase, barstar, PAT	<i>Bacillus amyloliquefaciens</i>
Herbicide resistance	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	<i>Agrobacterium tumefaciens</i> CP4
Herbicide resistance	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)	<i>Zea. mays</i>
Herbicide resistance	acetolactate synthase	chimera of 2 resistant AHAS genes (S4-Hr4)
Herbicide resistance	acetolactate synthase	chlorsulfuron tolerant line of <i>A. thaliana</i>
Herbicide resistance	acetolactate synthase	chlorsulfuron tolerant <i>Nicotiana tabacum</i>
Herbicide resistance	glyphosate oxidoreductase	<i>Ochrobactrum anthropi</i>
Herbicide resistance	Nitrilase	<i>Klebsiella pneumoniae</i> subspecies <i>ozanae</i>
Herbicide resistance	phosphinothricin N-acetyltransferase	<i>S. hygrosopicus</i>

Herbicide resistance	phosphinothricin N-acetyltransferase	<i>S. viridochromogenes</i>
Insect resistance	Cry1Ab delta-endotoxin (Btk HD-1)	<i>Bacillus thuringiensis subsp. kurstaki (Btk)</i>
Insect resistance	Cry1Ac delta-endotoxin	<i>Bacillus thuringiensis subsp. kurstaki (Btk)</i>
Insect resistance	cry1F delta-endotoxin	<i>Bacillus thuringiensis var. aizawai</i>
Insect resistance	Cry2Ab delta-endotoxin	<i>Bacillus thuringiensis</i>
Insect resistance	cry3A delta-endotoxin	<i>Bacillus thuringiensis subsp. Tenebrionis</i>
Insect resistance	cry3Bb1 delta-endotoxin	<i>Bacillus thuringiensis subsp. kumamotoensis</i>
Insect resistance	cry9c delta-endotoxin	<i>Bacillus thuringiensis subsp. Tolworthi</i>
Insect resistance	protease inhibitor	<i>Solanum tuberosum</i>
Male sterility	barnase ribonuclease	<i>Bacillus amyloliquefaciens</i>
Male sterility	DNA adenine methylase	<i>Escherichia coli</i>
Modified color	dihydroflavonol reductase	<i>Petunia hybrida</i>
Modified color	flavonoid 3p, 5p hydroxylase	<i>Petunia hybrida</i>
Modified color	flavonoid 3p, 5p hydroxylase	<i>Viola sp.</i>
Nicotine reduced	nicotinate-nucleotide pyrophosphorylase (carboxylating)	<i>Nicotiana tabacum</i>
Ripening delayed	1-amino-cyclopropane -1-carboxylic acid synthase	<i>Dianthus caryophyllus L.</i>
Ripening delayed	1-amino-cyclopropane-1-carboxylic acid deaminase	<i>Pseudomonas chlororaphis</i>
Ripening delayed	aminocyclopropane cyclase synthase	Tomato
Ripening delayed	polygalacturonase	Tomato
Ripening delayed	S-adenosylmethionine hydrolase	<i>E. coli bacteriophage T3</i>
Virus resistance	helicase	potato leafroll luteovirus (PLRV) orf 2
Virus resistance	replicase (RNA dependent RNA polymerase)	potato leafroll luteovirus (PLRV) orf 1
Virus resistance	viral coat protein	Cucumber mosaic cucumovirus
Virus resistance	viral coat protein	papaya ringspot potyvirus (PRSV)
Virus resistance	viral coat protein	potato potyvirus Y (PVY) strain O (common strain)
Virus resistance	viral coat protein	Watermelon mosaic potyvirus 2
Virus resistance	viral coat protein	Zucchini yellow mosaic potyvirus

Table 2: Data is derived from the Animal and Plant Health Inspection Services website (APHIS; <http://www.aphis.usda.gov/>).

5 Some specific crops have been broadly engineered to include the commercially advantageous traits listed above. For example, some of the more well-known transgenic traits, and the genes responsible for them, that have been introduced into use in oilseed rape production, are listed in Table 3, hereinbelow.

Table 3
Examples of primary advantageous genes inserted into oilseed rape for potentially commercial purposes in USA

<u>Type of gene/phenotype</u>	<u>Gene</u>	<u>APHIS #</u>
<u>Herbicide Resistance</u>		
CBI	CBI	03-254-03
Glyphosate	EPSP synthase	01-080-04
	glyphosate oxidoreductase	96-045-02
Glufosinate	CBI	00-023-05
	Phosphinothricin acetyl transferase	98-274-10
Bromoxynil	nitrilase	98-243-02
Sulfonylurea	acetolactate synthase	96-102-01
<u>Insect Resistance</u>		
Lepidopteran	CryIA(c)	02-312-02
	Trypsin inhibitor	99-098-05
	proteinase inhibitor II + CryIA(c)	94-326-01
<u>Disease resistance (fungal)</u>		
Cylindrosporium +Phoma	chitinase + glucanase	01-074-11
post harvest	coat protein	97-078-02
<u>Agronomic traits</u>		
Nitrogen metabolism altered	alanine amino transferase	03-276-04
Cold tolerance	cold regulated gene binding factor	01-066-07
Male sterility/fertility	CBI	03-254-03
	Barnase/Barstar	98-119-01
Yield increase	sucrose phosphate synthase	98-064-20
<u>Product Quality</u>		
Oil profile altered	Acyl CoA reductases, Acyl-ACP thioesterases, elongase, ketoacyl-ACP synthases	97-022-02
	Acyl-ACP-thioesterase + Delta 12saturase, Delta 9 + 15 desaturases	01-068-01
	Acetyl CoA carboxylase	99-067-02
	34 different genes	96-071-07
Lysine increased	Dihydropicolinate synthase	98-099-03
Pharmaceutical protein	CBI	96-215-01
Polymer	CBI	96-061-02
Industrial enzymes	CBI	93-048-02

5

The term "exogenous polynucleotide" as used herein, refers to any DNA or RNA molecule that is transformed (*i.e.*, integrates) into the genome of a target plant.

According to one preferred embodiments of this aspect of the present invention, such a polynucleotide comprises a nucleic acid sequence encoding a polypeptide (a gene product). The polypeptide encoded by the polynucleotide can be involved in the synthesis of a target polypeptide or nutrient (e.g., dihydropicolinate synthase), can be a regulatory polypeptide which is involved in the inhibition, degradation or inactivation of a target polypeptide or nutrient [e.g., *barstar* (GenBank Accession No. X15545; SEQ ID NO:40)] can regulate its production, stabilization or

activation (e.g. herbicide resistance such as glyphosate resistance) or can be a lethal product (e.g., ribosome inhibitor protein).

According to another preferred embodiments of this aspect of the present invention, such a polynucleotide is a DNA or an RNA that comprises a nucleic acid sequence encoding a regulatory agent (*i.e.*, an upregulation agent or a downregulation agent) which affects the level of gene expression. Non-limiting examples of such downregulation polynucleotides are antisense nucleic acid sequences, siRNA and the like. Non-limiting examples of upregulating polynucleotides are promoter sequences, enhancers and the like. Methods of inserting the exogenous polynucleotide into a cultivated plant are further described hereinbelow.

Thus, the exogenous polynucleotide of the present invention is inserted into the genome of the cultivated plant in a locus that is in high linkage disequilibrium with the *Ph1* locus. Once integrated in close proximity to the *Ph1* locus of the cultivated plant, the stable transgene introgression of the transgene is prevented, thus leaving the transgene in the target cultivated plant and avoiding introgression to homoeologous chromosomes of related, non-cultivated plants.

Stable transgene introgression can be also prevented using alternative approaches. For example, the exogenous polynucleotide of the present invention that is inserted into a locus being in linkage disequilibrium of the *Ph1* locus can be a suppressor of a product that is lethal to related, non cultivated plants. Such a suppressor can be for example, the *barstar*, which inhibits the action of *barnase* (a suicide gene encoding RNase which is lethal to plants over-expressing it).

Thus, according to another preferred embodiments of this aspect of the present invention, the exogenous polynucleotide comprises a nucleic acid sequence encoding a suppressor of a product lethal to the related, non-cultivated plant.

A product lethal to the related, non-cultivated plant can be any polynucleotide (DNA or RNA) or polypeptide that causes death of a plant harboring such a product. Preferably, the product lethal to the related, non-cultivated plant can be *barnase* (e.g., GenBank Accession No. M14442; SEQ ID NO:41).

Accordingly, a suppressor of such a product can be any polynucleotide or polypeptide which is capable of suppressing, inhibiting, downregulating or degrading the lethal product. Non-limiting examples of such suppressors include the gene encoding *barstar* (e.g., which is isolated from the *Bacillus amyloliquefaciens* species, GenBank Accession No. X15545; SEQ ID NO: 40) whose product suppresses the

action of the gene *barnase* (e.g., *barnase* isolated from *B. amyloliquefaciens* species, GenBank Accession No. M14442; SEQ ID NO: 41).

Once this configuration is utilized, the transgene of interest, which encodes the commercially desired trait, is preferably inserted into a second locus which segregates independently of the *Ph1* locus (*i.e.*, which is not linked to the *Ph1* locus or that is in linkage equilibrium with the *Ph1* locus).

Thus according to additional preferred embodiments of the present invention, the method according to this aspect of the present invention further comprising inserting a second exogenous polynucleotide into the second genetic locus of the transgenic cultivated plant, when the second genetic locus being in random association with the *Ph1* locus of the transgenic cultivated plant.

Preferably, the second exogenous polynucleotide comprises a nucleic acid sequence encoding a product lethal to the related, non-cultivated plant.

To prevent stable introgression of the transgene into the related, non-cultivated plant, the second exogenous polynucleotide preferably includes a second nucleic acid sequence encoding a transgene-of-interest.

It will be appreciated that to ensure the co-localization of the transgene with the lethal product, the nucleic acid sequences encoding the transgene and the lethal product are preferably conjugated in tandem a single nucleic acid construct.

Thus, under such configurations (*i.e.*, when the transgene forms a part of the same polynucleotide having the nucleic acid sequence encoding the lethal product), in case a stable genomic introgression occurs between the chromosomes of the transgenic cultivated plant and the chromosomes of the related, non-cultivated plant (*i.e.*, stable introgression of the transgene), the plants to which the transgene has introgressed is expected to die (due to the effect of the lethal product). On the other hand, the progeny of the transgenic cultivated plant, which undergoes homologous recombination, are protected from the effect of the lethal product due to the over-expression of the suppressor of the lethal product that is encoded by the exogenous polynucleotide inserted in the locus being in linkage disequilibrium with the *Ph1* locus.

The exogenous polynucleotide of the present invention (*i.e.*, the polynucleotide comprising the nucleic acid sequence of the transgene, the lethal product or the suppressor of the lethal product) can be engineered for plant expression using a suitable chimeric gene and transformation vector. A typical chimeric gene for

transformation into a plant includes a promoter region, a heterologous structural DNA coding sequences (e.g., the nucleic acid sequence of the transgene, the lethal product and the suppressor of the lethal product) and a 3' non-translated polyadenylation site. A heterologous structural DNA coding sequence means a structural coding sequence that is not native to the plant being transformed. Heterologous with respect to the promoter means that the coding sequence does not exist in nature in the same gene with the promoter to which it is now attached. Chimeric means a novel non-naturally occurring gene which is comprised of parts of different genes. It will be appreciated that the exogenous polynucleotide of the present invention can also utilize a natural promoter of a specific desired gene. In preparing the transformation vector, the various DNA fragments may be manipulated as necessary to create the desired vector. This includes using linkers or adaptors as necessary to form suitable restriction sites or to eliminate unwanted restriction sites or other similar manipulations that are known to those of ordinary skill in the art.

Promoters that are known or found to cause transcription of selected gene or genes in plant cells can be used to implement the present invention. Such promoters may be obtained from plants, plant pathogenic bacteria or plant viruses, or any other organism, and include, but are not necessarily limited to, strong constitutive promoter such as a 35S promoter [Odell et al (1985) Nature 313, 810-812], a 35S'3 promoter [Hull and Howell (1987) Virology 86, 482-493] and the 19S promoter of cauliflower mosaic virus (CaMV35S and CaMV19S), the full-length transcript promoter from the figwort mosaic virus (FMV35S) and promoters isolated from plant genes such as EPSP synthase, ssRUBISCO genes and promoters obtained from T-DNA genes of *Agrobacterium tumefaciens* such as the promoter of the nopaline synthetase gene ("PNOS") of the Ti-plasmid [Herrera-Estrella (1983) Nature 303, 209-213], the mannopine synthase promoter, or the promoter of the octopine synthase gene ("POCS" [De Greve et al (1982) J. Mol. Appl. Genet. 1 (6), 499-511]). Also useful can be expression in wound tissue, for example, using a second promoter which is a TR promoter such as the TRi' or TR2' promoter of the Ti-plasmid [Velten et al (1984) EMBO J. 3, 2723-2730]. Selective expression in green tissue can be achieved by using, for example, the promoter of the gene encoding the small subunit of Rubisco (European patent application 87400544.0 published Oct. 21, 1987, as EP 0 242 246). Promoters can be selected so that the transgenes are expressed in specific cells, such as petal cells, leaf cells or seed cells. All of these promoters have been used to create

various types of DNA constructs that have been expressed in plants. See, for example PCT publication WO 84/02913 (Rogers et al., Monsanto, herein incorporated by reference in its entirety). The particular promoter selected should be capable of causing sufficient expression to result in the production of an effective amount of the
5 respective proteins to confer the traits.

Particularly useful promoters for use in some embodiments of the present invention are fruit specific promoters and the full-length transcript promoter from the figwort mosaic virus (FMV35S). The FMV35S promoter is particularly useful because of its ability to cause uniform and high levels of expression in plant tissues.

10 The DNA sequence of a FMV35S promoter is presented in U.S. Pat. No. 5,512,466 and is identified as SEQ ID NO:17 therein. Other examples of fruit specific promoters include the E8, E4, E17 and J49 promoters from tomato (Lincoln *et al.*, 1988), as well as the 2A11 promoter as described in U.S. Pat. No. 4,943,674.

Tapetum and anther specific promoters can be used to isolate gene expression to
15 reproductive organs, to achieve, for example, male sterility. Promoters that show this specificity are well known. Especially useful are the tapetum-specific promoter Tap1 as described in Nacken et al. Sol. Gen. Genet. 229, 129-136, 1991), the tapetum specific promoters A9 (WO 92/11379), T29, PTA 29, PTA 26 and PTA13 as well as any promoter of a gene encoding a tapetum-specific mRNA hybridizable to the genes
20 TA29, TA26 or TA13 from which genes the PTA29, PTA26 and PTA13 promoters have been isolated (U.S. Patent Nos. 5,652,354 and 6,046,382 to Mariani et al), the anther specific promoters described in WO 92/18625, WO 90/08826 and European patent application EP 93810455.1, and the tapetum specific promoter MFS14 (WO 97/04116). Examples of female organ-specific promoters and sterility promoters are:

25 the style and/or stigma-specific promoters, such as PSTMG07, PSTMG08, PSTMG4B12 and PSTMG3C9, and the ovule-specific promoter corresponding to the cDNA clone pMON9608 as described in U.S. Pat. No. 5,633,441; as well as a promoter of a gene encoding i) a style-stigma specific or ii) an ovule-specific mRNA hybridizable respectively to i) a STMG-type style-stigma specific gene or ii) CDNA
30 clone pMON9608 of U.S. Pat. No. 5,633,441. Several other promoters are known in the art (see, e.g., McCormick et al. "Anther-Specific Genes: Molecular Characterization and Promoter Analysis in Transgenic Plants" in Plant Reproduction: From Floral Induction to Pollination, Lord et al. (ed.), 128-135, 1989; and Scott et al., 1992, The Plant Cell 4, 253, and U.S. Patent No. 6,603,064 to Van Dun) and as long

as they give specific expression in the reproductive system, choice of the promoter is not critical to the invention.

The 3' non-translated region contains a polyadenylation signal that functions in plants to cause the addition of polyadenylated nucleotides to the 3' end of an RNA sequence. Examples of suitable 3' regions are the 3' transcribed, non-translated regions containing the polyadenylation signal of the tumor-inducing (Ti) plasmid genes of *Agrobacterium*, such as the nopaline synthase (NOS) gene, and plant genes like the 7S soybean storage protein genes and the pea E9 small subunit of the RuBP carboxylase gene (ssRUBISCO).

The RNAs produced by a DNA construct of the present invention also preferably contain a 5' non-translated leader sequence. This sequence can be derived from the promoters selected to express the genes, and can be specifically modified so as to increase translation of the mRNAs. The 5' non-translated regions can also be obtained from viral RNA's, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5' non-translated sequence that accompanies the promoter sequence. Rather, the non-translated leader sequences can be part of the 5' end of the non-translated region of the native coding sequence for the heterologous coding sequence, or part of the promoter sequence, or can be derived from an unrelated promoter or coding sequence as discussed above.

In a preferred embodiment according to the present invention, the vector that is used to introduce the encoded proteins into the host cells of the plant comprises an appropriate selectable marker. In a more preferred embodiment according to the present invention the vector is a plant expression vector comprising both a selectable marker and an origin of replication. In another most preferred embodiment according to the present invention the vector is a shuttle vector, which can propagate both in *E. coli* (wherein the construct comprises an appropriate selectable marker and origin of replication) and be compatible for propagation or integration in the genome of the plant organism of choice.

According to some embodiments of the present invention, secretion of the protein or proteins out of the cell is preferred. In this embodiment the construct comprises a signal sequence to effect secretion as is known in the art. For some applications, a signal sequence that is recognized in the active growth phase will be most preferred. As will be recognized by the skilled artisan, the appropriate signal

sequence should be placed immediately downstream of the translational start site (ATG), and in frame with the coding sequence of the gene to be expressed.

The exogenous polynucleotide of the present invention is utilized to stably transform plant cells. In stable transformation, the exogenous polynucleotide of the present invention is integrated into the plant genome and as such it represents a stable and inherited trait.

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

The principle methods of causing stable integration of exogenous DNA into plant genomic DNA include two main approaches:

(i) *Agrobacterium*-mediated gene transfer: Klee et al. (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.

(ii) direct DNA uptake: Paszkowski et al., in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. et al. (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang et al. Plant Cell Rep. (1988) 7:379-384. Fromm et al. Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein et al. Bio/Technology (1988) 6:559-563; McCabe et al. Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990) 79:206-209; by the use of micropipette systems: Neuhaus et al., Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; glass fibers or silicon carbide whisker transformation of cell cultures, embryos or callus tissue, U.S. Pat. No. 5,464,765 or by the direct incubation of DNA with germinating pollen, DeWet et al. in Experimental Manipulation of Ovule Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels, W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

The *Agrobacterium* system includes the use of plasmid vectors that contain defined DNA segments that integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the *Agrobacterium* delivery system. A widely used approach is the leaf disc procedure which can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation. Horsch et al. in Plant Molecular Biology Manual A5, Kluwer Academic Publishers, Dordrecht (1988) p. 1-9. A supplementary approach employs the *Agrobacterium* delivery system in combination with vacuum infiltration. The *Agrobacterium* system is especially viable in the creation of transgenic dicotyledonous plants, but with the addition of suitable chemicals also works with monocotyledonous plants. Other bacteria related to *Agrobacterium* can perform the same function (Broothaerts, W. et al. (2005) Gene transfer to plants by diverse species of bacteria. Nature 433, 632 -633).

There are various methods of direct DNA transfer into plant cells. In electroporation, the protoplasts are briefly exposed to a strong electric field. In microinjection, the DNA is mechanically injected directly into the cells using very small micropipettes. In microparticle bombardment, the DNA is adsorbed on microprojectiles such as gold or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues.

Following stable transformation plant propagation is exercised. The most common method of plant propagation is by seed. It is preferred that the seed be produced on a transformed plant such that the regenerated plant has the identical traits and characteristics of the parent transgenic plant. Therefore, it is preferred that the transformed plant be regenerated by a process that provides a rapid, consistent reproduction of the transformed plants.

Regeneration is a multi-stage procedure that requires alteration of culture medium or growth conditions between stages. Thus, the regeneration process involves: Stage one, initial tissue culturing; stage two, tissue culture multiplication; stage three, differentiation and plant formation; and stage four, greenhouse culturing and hardening. During stage one, initial tissue culturing, the tissue culture is established contaminant-free. During stage two, the tissue samples grown in stage one are divided and grown into individual plantlets. At stage three, the transformed plantlets are transferred to a greenhouse for hardening where the plants' tolerance to light is gradually increased so that it can be grown in the natural environment.

It will be appreciated that the nucleic acid construct of the present invention (which includes the exogenous polynucleotide of the present invention) can be randomly inserted or directly targeted into the genome of the cultivated plant.

To directly target the integration of the nucleic acid construct of the present invention (which includes the exogenous polynucleotide of the present invention) into a specific desired locus (e.g., a locus that is in linkage disequilibrium with the *Ph1* locus, or a locus from the centromeric region of a chromosome), gene knock-in methods (also called site specific recombination) are employed. Thus, sequences homologous to regions upstream or downstream of the desired locus (e.g., the *Ph1* locus) are generated to enable recombination events between the vector and the target genomic region. Non-limiting examples of such sequences are provided in Table 1, hereinabove. It will be appreciated that in case the length of such sequences is insufficient to enable recombination, additional sequences can be uncovered using PCR with primers derived from the known sequence (e.g., from SEQ ID NO:13) and a random primer using methods well known in the art.

Such DNA constructs preferably include positive and negative selection markers and may therefore be employed for selecting homologous recombination events. One ordinarily skilled in the art can readily design a knock-in construct including both positive and negative selection genes for efficiently selecting transformed plant cells that underwent a homologous recombination event with the construct. Such cells can then be grown into full plants. Standard methods known in the art can be used for implementing knock-in procedures. Such methods are set forth in, for example, U.S. Pat. Nos. 5,487,992, 5,464,764. It will be appreciated that to increase the efficiency of the targeted insertion, a gene that promotes homologous pairing such as the yeast *RAD54* gene can also be engineered as part of the construct inserted into the plant, essentially as described elsewhere (Shaked, H., Melamed-Bessudo, C., Levi, A. A. 2005. High-frequency gene targeting in *Arabidopsis* plants expressing the *RAD54* gene. Proceedings of the National Academy of Sciences USA 102: 12265-12269) and references therein.

For example, the first exogenous polynucleotide of the present invention can include a constitutive promoter (e.g., 35S), the *barstar* gene (GenBank Accession No. X15545), a marker gene [e.g., hygromycin resistance (GenBank Accession No. E00287; SEQ ID NO: 42)], and two flanking regions that contain non coding regions specific to the long arm of wheat chromosome 5B, such as WPG90 (SEQ ID NO:46)

(Segal, G. et al., 1997, Theoretical and Applied Genetics 94, 968-970; for more information see: <http://wheat.pw.usda.gov/GG2/index.shtml>). This construct will target the long arm of 5BL in the vicinity of *Ph1*.

The second exogenous polynucleotide of the present invention can contain a constitutive promoter, such as 35S, the transgene of choice, such as glufosinate resistance (GenBank Accession No. M37389; SEQ ID NO: 43), a marker gene, such as *GUS* (GenBank Accession No. Y82477; SEQ ID NO: 44), and two flanking regions that contain non coding sequences specific to any wheat chromosome other than 5BL, such as WPG118 (; SEQ ID NO: 45) (Liu, B., et al., 2003, A chromosome-specific sequence common to the B genome of polyploid wheat and *Aegilops searsii*. Plant Systematics and Evolution 241, 959-965; Weissmann, S., et al., 2005. Sequence evidence for sporadic inter-generic DNA introgression from wheat into a wild *Aegilops* species. Molecular Biology and Evolution 22:2055-2062; for more information see <http://wheat.pw.usda.gov/GG2/index.shtml>). This construct can be targeted to any wheat chromosome except to the long arm of chromosome 5B. The two constructs can be simultaneously inserted into wheat callus by *Agrobacterium* transformation as described by Terada et al [Terada, R., et al., 2004, A large-scale *Agrobacterium*-mediated transformation procedure with a strong positive-negative selection for gene targeting in rice (*Oryza sativa* L.). Plant Cell Reports 22, 653-659].

For random insertion of the exogenous polynucleotide, a DNA construct comprising the gene-of-interest, for example the glyphosate resistance gene (SEQ ID NO:43) and a marker gene, such as *GUS* (SEQ ID NO:44) are inserted into immature wheat embryos using particle bombardment (Stoger, E., Williams, S., Keen, D., Christou, P. 1999. Molecular characteristics of transgenic wheat and the effect on transgene expression. Transgenic Research 7: 463-471). A screen for the desired location of the transgene in the vicinity of the *Ph1* locus on the long arm of wheat chromosome 5B is further performed using FISH analysis with a gene-specific probe (e.g., SEQ ID NO:43), essential as described in Stoger, E., et al., 1999 (Molecular characteristics of transgenic wheat and the effect on transgene expression. Transgenic Research 7: 463-471) which is fully incorporated herein by reference. Following FISH analysis, the stained chromosomes are photographed (e.g., using a camera connected to a microscope or an image analysis system) and the position of the labeled chromosome is recorded. Prior to C-banding, the fluorescent probe is removed by incubating for 4 minutes the chromosomes specimen in a warm solution

(at 60 °C) of 70 % formamide in 300 mM NaCl, 30 mM NaCitrate (2XSSC), followed by dehydration in a series of cold (at -20 °C) ethanol (*i.e.*, 70, 95 and 100 %). Following dehydration the chromosome specimen is subjected to a standard C banding essentially as described in Rodriguez, S., et al. 2000 (Chromosome structure of *Triticum timopheevii* relative to *T. turgidum*. Genome 43: 923-930). Embryos in which the gene-of-interest has been inserted into 5BL near the *Ph1* locus are further selected.

Once a transgenic plant tissue that contains an expression vector has been obtained, transgenic plants are regenerated from this transgenic plant tissue. The term "regeneration" as used herein, means growing a whole plant from a plant cell, a group of plant cells, a plant part or a plant piece. Transformed plant cells that are derived by any of the above transformation techniques are cultured to regenerate a whole plant which possesses the transformed genotype and thus the desired phenotype. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with the nucleic sequence of interest. Regeneration techniques for regenerating plants from plant callus, explants, organs, or parts thereof are described generally in Klee et al. Ann. Rev. of Plant Phys. 38:467-486 (1987). The culture media will generally contain various organic components including vitamins, sugars, and plant hormones, such as auxin and cytokinins, as well as inorganic salts. The regenerated plants are transferred to standard soil conditions and cultivated in a conventional manner.

Confirmation of the transgenic nature of the cells, tissues, and plants may be performed by PCR analysis, antibiotic or herbicide resistance, enzymatic analysis and/or Southern blots to verify transformation. Progeny of the regenerated plants may be obtained and analyzed to verify whether the transgenes are heritable. Heritability of the transgene is further confirmation of the stable transformation of the transgene in the plant. Progeny may be sexually or asexually derived progeny.

After the desired genes or construct is stably incorporated into regenerated transgenic plants, they can be transferred to other plants by sexual crossing. The plants are then grown and harvested using conventional procedures.

Thus, the teachings of the present invention can be used to prevent DNA introgression between homoeologous chromosomes.

Studies have shown that recombination in wheat occurs mainly in the distal one-third of the chromosomes (Akhunov *et al.* 2003; Lukaszewski and Curtis 1993). While further reducing the present invention to practice, the present inventors have uncovered that insertion of the transgene near the centromere will reduce the probability of stable transgene introgression.

Thus, according to yet an additional aspect of the present invention there is provided a method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant. The method is effected by inserting an exogenous polynucleotide near a centromeric region of a chromosome of the transgenic cultivated plant thereby preventing the stable transgene introgression into the related, non-cultivated plant.

As used herein, the phrase "centromeric region of a chromosome" refers to the nucleic acid sequence encompassed by the centromere of the chromosome of the transgenic cultivated plant. Preferably, the method is effected by inserting the exogenous polypeptide near a sub-centromeric region of the chromosome of the transgenic cultivated plant. The phrase "sub-centromeric" refers to a portion of the centromere of the chromosome, preferably, a portion that includes 0.5 Morgan unit on each side of the centromere.

Tables 4 and 5 hereinbelow, list genes, markers and loci which can be used for targeting the exogenous polynucleotide to the sub-centromeric region of wheat chromosomes.

Table 4
Non-coding sequences in sub-centromeric regions

SEQ ID No.	Name	Chromosome	coding	Distance from the centromere (break point interval)	GenBank accession No.
39	BARC58	5BL	No	C-5BL6-0.29	BV211574
17	BARC89	5BL	No	C-5BL6-0.29	BV211400
18	BARC28	1AS	No	C-1AS1-0.47	BV211769
19	BARC187	1BL	No	C-1BL6-0.32	BV211754
20	BARC99	1DL	No	C-1DL4-0.18	BV211579
21	BARC152	1DS	No	C-1DS3-0.48	BV211514
22	BARC208	2AS	No	C-2AS5-0.78	BV211645
23	BARC220	2AL	No	C-2AL1-0.85	BV211458
24	BARC18	2BS	No	C-2BS1-0.53	BV211636
25	BARC228	2DL	No	C-2DL3-0.49	BV211470
26	BARC57	3AL	No	C-3AL3-0.42	BV211789
27	BARC1077	3BL	No	C-3BL2-0.22	BV211468
28	BARC139	3BS	No	C-3BS1-0.33	BV211305
29	BARC1052	4AS	No	4AS1-0.20-0.63	BV211382
30	BARC1001	4BS	No	C-4BS4-0.37	BV211314
31	BARC155	5AL	No	C-5AL12-0.35	BV211515
32	BARC207	5AL	No	C-5AL12-0.35	BV211644
33	BARC110	5DL	No	C-5DL1-0.60	BV211405
34	BARC107	6AL	No	C-6AL4-0.55	BV211404
35	BARC211	6BS	No	C-6BS5-0.76	BV211437
36	BARC175	6DL	No	C-6DL6-0.29	BV211752
37	BARC1005	7AS	No	C-7AS8-0.45	BV211709
38	BARC63	7BS	No	BARC63	BV211683

Table 4: Non-coding sequences from a sub-centromeric region of the chromosome. The distance from the centromere is provided using deletion lines; the distance from the centromere is represented by the remaining fraction of the specific chromosomal arm.

Table 5
Markers tightly linked to the centromere

Marker	Chromosome	Map
GBXGX74	1A	Wheat-Composite2004-1A
CDO127	1B	Wheat-Composite2004-1B
GBXG542	2A	Wheat-Composite2004-2A
GBX4986	2A	Wheat-Composite2004-2A
GBXG553	2B	Wheat-Composite2004-2B
GBXR635	2D	Wheat-Composite2004-2D
GBXG406	3A	Wheat-Composite2004-3A
GBXG392	3B	Wheat-Composite2004-3B
GBXG305	3D	Wheat-Composite2004-3D
FBA211	4A	Wheat-Composite2004-4A
GBXR657	4B	Ta-Synthetic/Opata-gbx-4B
GBXG129	4D	Wheat-Composite2004-4D
CDO785	5A	Wheat-Composite2004-5A
MWG561	5B	Ta-Synthetic/Opata-5B
FBA137	5D	Wheat-Composite2004-5D
GBXG728	6A	Wheat-Composite2004-6A
GBXG138	6B	Wheat-Composite2004-6B
FBB231	6D	Ta-Synthetic/Opata-SSR2-6D
GBXG732	7A	Wheat-Composite2004-7A
BCD178	7B	Wheat-Composite2004-7B
BCD707	7D	Ta-Synthetic/Opata-7D

Table 5: Data from <http://wheat.pw.usda.gov/GG2/index.shtml>

According to one preferred embodiment of this aspect of the present invention,
 5 the exogenous polynucleotide of the present invention can be any of the
 polynucleotides set forth by SEQ ID NOs:17-39.

It will be appreciated that such a method can be used in most species
 (including those of the tribe *Triticeae*), except for in those species (e.g., family
 Liliaceae others) within which there is a high incidence of recombination of genes
 10 near the centromere.

Following is a list of species in which recombination occurs near the
 centromeric region (*i.e.*, species in which the transgene should not be inserted into the
 centromeric region):

Several species of *Fritilaria* (Darlington, C. D. 1965. Cytology. Part I: recent
 15 advances in cytology, 1937: Part II: Recent advances in cytology, 1937-1964. J. & A.
 Churchill ltd, London).

Several species of *Bellevalia* [Gopal-Ayengar, A. R. 1941. The origin and
 behaviour of chiasmata in *Bellevalia* species. (abstr.) Amer. J. Bot. 28: 3s]

Several species of *Allium* [Levan, A. 1933. Cytological studies in *Allium*, IV.
 20 *Allium fistulosum*. Svensk Bot. Tidskr. 27: 211-232; Levan, A. 1935. Cytological
 studies in *Allium*, VI. The chromosome morphology of some diploid species of
Allium. Hereditas 20: 289-330; Levan, A. 1940. Meiosis of *Allium porrum*, a
 tetraploid species with chiasma localisation. Hereditas 26: 454-462; Ved Brat, s.
 1965. Genetic system in *Allium*. III. Meiosis and breeding systems. Hereditas 20: 325-
 25 339; Koul, A. K. and R. N. Gohil, 1970. Cytology of the tetraploid *Allium*
ampeloprasum with chiasma localisation. Chromosoma 29: 12-19].

According to one preferred embodiments of the this aspect of the present
 invention, the exogenous polynucleotide is inserted into the genome of the target
 cultivated plant in a locus which is in linkage disequilibrium with the *Ph1* (as
 30 described hereinabove) and which is also included in the centromeric region
 (preferably the sub-centromeric region) of the targeted chromosome (e.g.,
 chromosome 5 of subgenome B of *T. aestivum* or *Triticum turgidum*). For example,
 the exogenous polynucleotide encoding a transgene of interest (e.g., glyphosate
 resistance, GenBank Accession No. M37389) is inserted using homologous

recombination to the centromeric sequences (e.g., SEQ ID NO: 17 and/or 39), that are near the *Ph1* locus and also near the centromere.

Similarly, the exogenous polynucleotide having the nucleic acid sequence encoding the suppressor of the lethal product (e.g., *Barstar*, SEQ ID NO: 40) can be inserted within the sub-centromeric region of the target chromosome. Preferably, it can be inserted in a locus which is in linkage disequilibrium with the *Ph1* locus and yet within the sub-centromeric region of the chromosome (e.g., SEQ ID NOs: 17 and/or 39). As is mentioned before, under such a configuration, the second exogenous polynucleotide which includes the transgene and the lethal gene is inserted in a locus which independently segregates from the first locus (which is near the centromere and *Ph1*), such a second locus can be on another chromosome.

As used herein the term "about" refers to $\pm 10\%$.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions; illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include cytogenetic, molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Chromosome Engineering in Plants" Parts A and B, Gupta PK and Tsuchiya T. Eds., (1991); "Molecular Cloning: A Laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., Ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (Eds.) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor

Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., Ed. (1994); "Oligonucleotide Synthesis" Gait, M. J., Ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., Eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

HOMOEOLOGOUS INTROGRESSION OF DNA SEQUENCES FROM THE GENOME OF *T. AESTIVUM* (CULTIVATED WHEAT) INTO THE GENOME OF *AE. PEREGRINA*

Materials and Experimental Methods

Amplified fragment length polymorphism (AFLP) analysis - AFLP analysis was performed essentially according to Vos et al. 1995 [Vos, P.H.R., Bleeker, M., Reijans, M., Van der Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. AFLP: a new technique for DNA fingerprinting. (1995). Nucleic Acids Research 23 (21): 4407-4414] except that the primers were labeled with one of the fluorescent dyes FAM (6-carboxyfluorescein), HEX (4, 7, 2', 4', 5', 7'-hexachloro-6-carboxyfluorescein), or TET (4, 7, 2', 7'-tetrachloro-6-carboxyfluorescein) (Eisenberg Bros. Ltd.) and following electrophoresis, the gel was scanned using the appropriate laser with a Typhoon™ 9400 scanner (Amersham Bioscience) at medium sensitivity and the intensity of the gel digital image was adjusted using the ImageQuant™ software. Ten primer combinations were randomly chosen for the analysis. The selective nucleotides and the primer dye are listed in Table 6, hereinbelow.

Table 6
Primers used for AFLP analysis

<i>MseI</i> selective nucleotides	<i>EcoRI</i> selective nucleotides
GATGAGTCCTGAGTAACTG (SEQ ID NO:1)	GACTGCGTACCAATTCAC (FAM) (SEQ ID NO:8)
GATGAGTCCTGAGTAACTT (SEQ ID NO:2)	GACTGCGTACCAATTCACA (TET) (SEQ ID NO:9)
GATGAGTCCTGAGTAACTT (SEQ ID NO:2)	GACTGCGTACCAATTC AAC (HEX) (SEQ ID NO:10)
GATGAGTCCTGAGTAACTT (SEQ ID NO:2)	GACTGCGTACCAATTCACC (FAM) (SEQ ID NO:11)
GATGAGTCCTGAGTAACAG (SEQ ID NO:3)	GACTGCGTACCAATTCACA (TET) (SEQ ID NO:9)
GATGAGTCCTGAGTAACAT (SEQ ID NO:4)	GACTGCGTACCAATTCACA (TET) (SEQ ID NO:9)
GATGAGTCCTGAGTAACTA (SEQ ID NO:5)	GACTGCGTACCAATTCACA (TET) (SEQ ID NO:9)
GATGAGTCCTGAGTAACTC (SEQ ID NO:6)	GACTGCGTACCAATTCACA (TET) (SEQ ID NO:9)
GATGAGTCCTGAGTAACAG (SEQ ID NO:7)	GACTGCGTACCAATTCACC (FAM) (SEQ ID NO:11)
GATGAGTCCTGAGTAACAG (SEQ ID NO:7)	GACTGCGTACCAATTCACG (HEX) (SEQ ID NO:12)

Table 6: The selective nucleotides combination of primers that were randomly chosen to detect, by AFLP, introgression from *T. aestivum* into *Ae. peregrina*. The dye of the *EcoRI* primers is in brackets

Cytogenetic analysis of hybrids and successive backcross generations - Root tips from F₁ hybrids and backcross seedlings were collected and placed in vials containing cold water, placed in crushed ice, refrigerated for 30 hours and then transferred into a fixative solution of 3 ethanol: 1 acetic acid. The fixative solution was changed several times during the first week. The material was kept at 4 °C until analyzed. Anthers from mature plants were placed immediately after collection in a fixative solution. Fixed root tips or anthers were placed on a glass slide with a drop of 2 % acetocarmine, heated over a moderate flame for several seconds and then squashed. The number of chromosomes (at mitosis) and bivalents (at meiosis) was determined by examination of the slides with an Olympus inverted system microscope IX, and photographed by an Imago CCD camera with TiLLvisION 3.3 software (T. I. I. L Photonics).

Experimental results

Fifteen BC₃F₂ plants were analyzed. All of them had 2n = 28 chromosomes (14 bivalents at first meiotic metaphase). A total of 17 amplified fragment length polymorphism (AFLP) fragments introgressed from *T. aestivum* into the BC₃F₂ plants: 2 bands from the B genome of *T. aestivum* (Table 7 herein below, and Figure 1), 2 bands from the A genome (Table 7 and Figure 2), and 7 from the D genome

(Table 7 and Figure 2) of *T. aestivum*. The origin of 6 additional introgressed bands was not determined (Table 7, Figure 3). Although 17 bands introgressed from *T. aestivum* into *Ae. peregrina*, it is not necessarily certain that these bands result from 17 separate recombination events. It is possible that the introgressed bands that originated from a certain genome of *T. aestivum* are all localized on the same large fragment that introgressed into *Ae. peregrina*. If the relative size of the different chromosome arms of BC₃F₂ plants are ignored, each chromosome arm in these plants constitutes about 1.78 % of the plant's genome *i.e.*, 1/(28 chromosomes x two arms each). If the three putative events of recombination result in the exchange of three chromosome arms between the chromosomes of the parental species, then the introgressed bands are expected to constitute about 5.36 % of the bands scored for BC₃F₂ plants. Interestingly, the 17 introgressed bands constitute 5.39 % of all the bands scored for BC₃F₂ plants (Table 8, herein below). Since the 17 bands originated from three different genomes of *T. aestivum*, it is more than likely that these bands introgressed by at least three events of recombination.

Table 7
Introgressed bands from *T. aestivum* into *Ae. peregrina*

<i>The genomic origin of the band in wheat</i>	<i>No. of introgressed bands</i>	<i>No. of segregating bands</i>	<i>Minimal no. of recombination events</i>
B	2	1	2
A	2	0	1
D	7	4	2
Not determined	6	2	-
Total	17	7	5

Table 7: Seventeen bands introgressed into *Ae. peregrina* from all three sub genome of *T. aestivum*. Segregation of introgressed bands from the same wheat genome origin indicates more than one events of recombination. The data indicate at least two recombination events between chromosomes of *Ae. peregrina* and those of the wheat genome D, at least two with those of wheat genome B, and at least one with those of wheat genome A.

Table 8
Introgression of *T. aestivum* DNA in stabilized 28 chromosomes BC_3F_2 plants

	<i>F</i> ₁		BC_3F_2		<i>Ae. peregrina</i>	<i>T. aestivum</i>
	% of parental unique bands	Band sharing	% of parental unique bands	Band sharing	Band sharing	Band sharing
<i>Ae. peregrina</i>	17.2	0.8	24.8	0.9	-	0.7
<i>T. aestivum</i>	19.0	0.8	5.4	0.7	0.7	-

Table 8: Introgression of *T. aestivum* DNA in stabilized 28 chromosomes BC_3F_2 plants. Similar values of band sharing were found between hybrids of *T. aestivum* and *Ae. peregrina* with the two parental species. The percentage of *Ae. peregrina* unique bands was five time higher than the percentage of *T. aestivum* unique bands in BC_3F_2 plants.

Three of the bands segregated among the 15 BC_3F_2 plants (that are all offspring of the same BC_3 plant). They were missing from 1/3 to 1/4 of the 15 plants and were present in 2/3 – 3/4 of them (see an example in Figure 4). One of the bands originated from the D genome of wheat; the origin of the other two was not determined. Since the other 6 bands that introgressed from the D genome of wheat did not segregate, it is possible that two separate segments introgressed from the D genome of wheat. In this case it is more than likely that at least four recombination events occurred in the process of these crosses.

Seven of the 17 introgressed bands showed segregation. They were missing from one or both tested BC_3 plants, but were present in all or some of the BC_3F_2 plants. Five of these bands originated from the D genome of wheat, one from the B genome and the origin of the seventh band was not determined (Table 7, hereinabove). These results further support the assumption of two separate introgressions from the D genome of wheat, since all BC_3 plants are offspring of the same BC_2 plant. It indicates that one of the fragments introgressed from the D genome has reached homozygosity and thus does not segregate but the other segments have not reached homozygosity and are still segregating. In addition, since one band of the B genome segregated among BC_3 plants and the other did not it is possible that two separate segments introgressed from the B genome as well. That brings the total number of possible recombination events in the process of these crosses to at least five.

Determination of chromosome number in the *F*₁ hybrids - To ascertain that the seeds collected from the parental *Ae. peregrina* plants are indeed *F*₁ hybrids, root

tips were collected from both plants, fixed, stained with acetocarmine, and then squashed on a glass slide to separate the chromosomes. Both plants had the expected number of 35 chromosomes (14 of chromosomes of *Ae. peregrina* + 21 chromosomes of *T. aestivum*) (Figure 6). These results demonstrate that these plants are hybrids resulting from the cross between *Ae. peregrina* and wheat.

Stabilization of chromosome number in backcross generations of crosses between Ae. peregrina and T. aestivum - Hybrid plants exhibited the expected number of 35 chromosomes (14 of chromosomes of *Ae. peregrina* + 21 chromosomes of *T. aestivum*) (Figure 6). BC₃ plants exhibited between 29 to 32 chromosomes.

This indicates a tendency of the chromosome number to stabilize at 28 chromosomes through backcrossing to *Ae. peregrina*. Three BC₃ plants contained trivalents at meiosis (Figure 7b). This may indicate a possible translocation from *T. aestivum* into *Ae. peregrina*. One (or two) of the homologous chromosomes of *Ae. peregrina* may contain a wheat segment homologous to that of the third chromosome (possibly a wheat chromosome) attached to the bivalent. One of the BC₃ plants (No. 49) was chosen for further analysis. This plant exhibited 29 chromosomes with 14 bivalents and one univalent. Thirty four of this plant's seeds were planted. Root tips from these BC₃F₂ plants were collected, and their chromosome numbers were determined. Five BC₃F₂ plants had 29 chromosomes and 29 plants had 28 chromosomes (Figure 7a). In meiosis, all the BC₃F₂ plants with 28 chromosomes had 14 bivalents. Interestingly, these BC₃F₂ plants had a significantly ($P = 1.07 \times 10^{-46}$) higher number of rod bivalents (range 3-7, average 4.03, Figure 7c) compared to their wild parental species *Ae. peregrina* (range 1-4, average 2.01, Figure 7d). As recombination tends to occur at higher rates towards the terminal ends of chromosome arms (Akhunov, E.D. et al., 2003, The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res. 13: 753-763), this may indicate introgression of wheat segments into the chromosomes of *Ae. peregrina* and thus the loss of homology between the edges of the homologous chromosomes.

Analysis and Discussion

Many publications in recent years focused on the formation of crop x wild inter-generic hybrids, and assumed that introgression between homoeologous genomes is mainly the result of homoeologous pairing in the F₁ hybrid (Guadagnuolo et al. 2001a; Hegde and Waines 2004; Seefeldt et al. 1998; Snyder et al. 2000; Wang

et al. 2001; Zemetra *et al.* 1998). Although formation of hybrids is crucial for the occurrence of introgression, inter-specific and inter-generic wheat F₁ hybrids are characterized by low homoeologous pairing. For example Jauhar and Peterson (2001; Hybrids between durum wheat and *Thinopyrum junceiforme*: Prospects for breeding for scab resistance. Euphytica. 118: 127–136.) Reported an average of 2 bivalents per cell between the chromosomes of durum wheat and *Thinopyrum junceiforme*. Ananthawat-Jonsson and Bodvarsdottir (1998; Chromosome Research 6: 339-343) reported 1 bivalent per cell between the chromosome of *T. aestivum* and *Leymus arenarius* and *L. mollis*). This is due to the presence of the *Ph1* gene that suppresses pairing between homoeologous chromosomes (Wall *et al.* 1971, Genetical Research 18: 329-339). The results described hereinabove, indicate the transfer of at least 5 different fragments from wheat into *Ae. peregrina* i.e. five recombination events (Table 4, hereinabove). This is ten times higher than the average number of bivalents per cell observed in hybrids between *T. aestivum* cv. Chinese Spring and *Ae. peregrina* TKE02 (Ozkan and Feldman 2001, Genome 44: 1000-1006.; Sears 1977, Canadian Journal of Genetics and Cytology 19: 585-593). Farooq *et al.* reported an increase of such magnitude in hybrids between *T. aestivum* and *Ae. peregrina* in the absence of *Ph1* (Farooq *et al.* 1990, Genome 33: 825-828). However, all the F₁ hybrids of the present study contained 21 wheat chromosomes (Figure 6) including chromosome 5B with *Ph1*. Hence, it is very unlikely that all the five putative recombination events that transferred wheat bands into *Ae. peregrina* occurred in the F₁ hybrids. Indeed, not all backcross plants contain *Ph1* (Figure 5). The low level of pairing in the F₁ hybrid causes chromosomes to segregate at random. An unpaired chromosome (univalent) has a probability of only 0.25 to be present in gametes (due to random segregation in the two meiotic divisions) (Morris and Sears 1967). F₁ hybrids of wide crosses with wheat are highly male sterile (Wang *et al.* 2001), meaning that most (75 %) BC₁ plants produced may not carry *Ph1*. As a result, all progeny of these *Ph1*-lacking plants also will not contain *Ph1*. Homoeologous pairing may thus sharply increase in these backcross progeny compared to the F₁ hybrids (Figures 4 and 5). Considering the facts that at least five introgressions in backcross plants all originated from a single F₁ plant; the probability for homoeologous pairing in the parental F₁ hybrid is extremely low, and the probability for homoeologous pairing in backcross plants lacking *Ph1* is high, it can be concluded

that most introgression events from *T. aestivum* to *Ae. peregrina* discovered in the present study occurred in backcross rather than in F₁ hybrid plants (Figures 4 and 5).

Since there is no information available on a homolog *Ph1* gene for species other than *Triticum*, the transition of *Ph1* to backcross plants is crucial to the prevention of homoeologous pairing and thus to the transfer of the transgene in backcross plants.

EXAMPLE 2

CHROMOSOMAL PLACEMENT AS A FAILSAFE TO PREVENT TRANSGENE MOVEMENT TO THE WILD POPULATION

Placement on chromosomes belonging to one of the A, B, or D genomes has been suggested as a possible failsafe mechanism for transgenic wheat. It has been suggested that placing the transgene on a wheat genome not shared by the neighboring wild or weedy relatives will reduce the risk of transgene movement (compared to genes on homologous chromosomes) because homoeologous chromosomes rarely pair in the F₁ hybrid (Gressel 2000; Wang *et al.* 2001). However, low homoeologous pairing occurs only in hybrid and backcross plants that contain *Ph1* but may increase sharply in backcross plants not containing *Ph1*. *Ph1* specifically prevents the promiscuous pairing of homoeologous chromosomes, preventing recombination within the three genomes of wheat (in the absence of homologous chromosomes) and with related species (Okamoto, M., 1957; Riley, R. & Chapman, V. 1958; Sears, E. R. & Okamoto, M. 1958; Sears, E. R. 1976). *Ph1* is located in the middle of the long arm of chromosome 5B (5BL) (Jampates, R. & Dvorak, J. 1986), about 1 centi Morgan (cM) from the centromere (Sears, E. R., 1984). *Ph1* (or a *Ph1* homolog) has not been found to occur in other Graminae where it had been actively sought except in *Triticum*, hence if *Triticum Ph1* is not present in the backcross plant homoeologous pairing is not suppressed. Consequently, a transgene placed on a genome homoeologous to that of the wild relative may not introgress in the hybrid plant but has a higher probability of introgressing in backcross generations (Figure 4).

Oilseed rape (*Brassica napus*, 2N = 38, AACC) another polyploid crop, has the *PrBn* gene that stringently controls the extent of meiotic pairing in haploids (Jenczewski, E. et al., 2003). *PrBn* is somewhat different from *Ph1* as *Ph1* prevents

homoeologous pairing at both haploid and diploid stages while *PrBn* controls pairing only in haploids. A system suppressing homoeologous pairing in the diploid stage of oilseed rape probably evolved long after the formation of the allopolyploid, as recently synthesized allopolyploids that mimic the original one have high rates of homoeologous recombination (Udall, J. A., et al., 2005).

When wheat with *Ph1* gene hybridizes with a wild polyploid species there is little or no homoeologous pairing in the F_1 , as long as *Ph1* is watchfully present as a chaperon. As an unpaired chromosome has a probability of only 0.25 of being present in the gametes (due to random segregation in the two meiotic divisions) (Morris, R. and Sears, E. R., 1967), about 75 % of the progeny resulting from the backcross of F_1 to the wild parent will lack wheat chromosome 5B, and consequently, *Ph1* [Figure 5, fail mechanism (a)]. In these plants single wild and wheat chromosomes will promiscuously homoeologous pairing and recombine with abandon, transferring genes with impunity.

The present inventors uncovered methods that significantly reduce the probability of homoeologous transgene introgression and stabilization in backcross generations of the transgene in the wild or weedy population.

I. A transgene is located in proximity to the centromere (in a region which is not subject to recombination) - As discussed hereinabove, recombination events in wheat occur mainly in the distal one-third of the chromosomes (Akhunov *et al.* 2003; Lukaszewski and Curtis 1993). Therefore, release of transgenic wheat cultivars with the transgene located in close proximity to the centromere would significantly reduce the chance of homoeologous transgene movement. It will be appreciated that such a method can be used in most species (including Triticeae), except than in those species (e.g., family Liliaceae and others) within which there is a high incidence of recombination of genes near the centromere.

Following is a list of species in which recombination occurs within the sub-centromeric region (i.e., species in which there would be no advantage if the transgene is inserted into the sub-centromeric region):

Several species of *Fritilaria* (Darlington, C. D. 1965. Cytology. Part I: recent advances in cytology, 1937: Part II: Recent advances in cytology, 1937-1964. J. & A. Churchill Ltd, London).

Several species of *Bellevalia* [Gopal-Ayengar, A. R. 1941. The origin and behaviour of chiasmata in *Bellevalia* species. (abstr.) Amer. J. Bot. 28: 3s]

Several species of *Allium* [Levan, A. 1933. Cytological studies in *Allium*, IV. *Allium fistulosum*. Svensk Bot. Tidskr. 27: 211-232; Levan, A. 1935. Cytological studies in *Allium*, VI. The chromosome morphology of some diploid species of *Allium*. Hereditas 20: 289-330; Levan, A. 1940. Meiosis of *Allium porrum*, a tetraploid species with chiasma localisation. Hereditas 26: 454-462; Ved Brat, s. 1965. Genetic system in *Allium*. III. Meiosis and breeding systems. Hereditas 20: 325-339; Koul, A. K. and R. N. Gohil, 1970. Cytology of the tetraploid *Allium ampeloprasum* with chiasma localisation. Chromosoma 29: 12-19].

II. A transgene is located in tight linkage to the *Ph1* locus (gene) which inhibits recombination of homoeologous chromosomes - *Ph1* is located on the long arm of wheat chromosome 5B (5BL) (Okamoto 1957) and strongly suppresses homoeologous pairing when present. If the transgene is located on this chromosome arm the probability of the transgene to transfer into a homoeologous genome is vastly reduced even in backcross plants. *Ph1* will be present in the backcross plant with the closely linked transgene and thus prevent homoeologous pairing and the transfer of the transgene into the homoeologous chromosomes of the other species.

Using the novel systems of targeted introgression (also called, confusingly homologous recombination by molecular biologists) (Puchta, H., 2002; Reiss, B., 2003; Li, H. Q. & Li, M. R., 2004), it is possible to insert the transgenes of choice in close proximity to *Ph1* on chromosome arm 5BL. Thus, the transgene of choice will remain genetically linked with watchful *Ph1*, and will segregate with it in the F₁ hybrid and backcrosses, and thus not introgress into the chromosomes of wild/weedy relatives [Figure 5, failsafe mechanism (b)]. During backcrosses with the wild/weedy species, the excess wheat chromosomes are selectively eliminated due to lagging during meiotic anaphases (Wang, Z. N., et al., 2001). Chromosome arm 5BL with the *Ph1* gene and the linked transgene will be retained in a small proportion of offspring only as long as the transgene confers a selective advantage, e.g. when the transgene is for a herbicide resistance, and the particular herbicide is used. In seasons where other herbicides are used, the selective disadvantage will eliminate 5BL. Such a solution, especially if coupled with other solutions, such as mitigating genes, could considerably lower the risk of stable transgene introgression between wheat and its relatives. Indeed, when *Ph1* is finally isolated, it too could be used as a mitigator gene, no longer necessitating targeted insertion (US Pat. Appl. No. 20040172678).

It will be appreciated that release of cultivars with the transgene located in the sub-centromeric region of the long arm of chromosome 5B is preferred. In this way even if the chromosome containing the transgene segregates into a backcross plant, and persists in 2-3 more generations until stabilization of the chromosome number, the transgene will not be able to transfer into the homoeologous genome and stabilize in the wild population. Methods such as site-specific gene integration (knock-in) (Srivastav and Ow, 2004) assist in creating wheat lines with specific insertion sites in the vicinity of *Ph1* that would allow the insertion of any desired transgene as well as its safe release.

Following are sequences that can be used for targeted insertion of the transgene into the sub-centromeric region of a chromosome and for targeting the insertion in tight linkage to the *Ph1* gene: BARC58 (non-coding; at break point interval C-5BL6-0.29; GenBank Accession No. BV211574; SEQ ID NO:39), and BARC89 (non-coding; at break point interval C-5BL6-0.29; GenBank Accession No. BV211400; SEQ ID NO:17).

EXAMPLE 3

AN ADDITIONAL SOLUTION TO PREVENT ALLOPOLYPLOID CROP GENE INTEGRATION INTO WILD/WEEDY RELATIVES

An additional way to curtail unwanted stable introgression of a wheat transgenes into wild or weedy relatives also utilizes insertion on chromosome 5BL, while providing another layer of protection. The transgene of choice (e.g., the transgene of commercial significance of which introgression is undesired) is inserted together with a suicide gene on any chromosome arm other than 5BL, and inserting a gene encoding a suppressor of the suicide gene product on chromosome arm 5BL [Figure 5, failsafe mechanism (c)]. The linkage between the suicide-suppressor gene and *Ph1* on 5BL will prevent the transfer of the suppressor to a wild chromosome and consequently, the establishment of this gene in the wild population. The suicide gene can encode any heterologous protein that is toxic to plants possessing it. Such a gene is *barnase*, isolated from *Bacillus amyloliquefaciens* (GenBank Accession No. M14442), which encodes a RNase that destroys RNA (Mariani, C. et al., 1992). The suicide-suppressor gene can be any gene that encodes a heterologous protein that inactivates either the suicide gene or the toxic protein encoded by the suicide gene.

Barstar is an intracellular suppressor of *barnase* that binds the *barnase* encoded protein and blocks its active site (Korchuganov, D. S. et al., 2004). The gene encoding *barstar* protein also was isolated from *B. amyloliquefaciens* (Hartley, R. W. 1989) (GenBank Accession No. X15545). Any backcross progeny having the tandem transgene of choice - suicide gene, but are without the suppressor of the suicide gene will die. Due to the presence of *Ph1*, 5BL will not homoeologously pair with chromosomes in the wild or weedy relative, and like extraneous chromosomes that are unpaired, 5BL will be eliminated during the continuous backcrossing to the wild or weedy parents.

It is appreciated that *Ph1* will prevent homoeologous recombination only in the absence of a *Ph1* suppressor gene such as that present in several diploid species, e.g., *Ae. speltoides*, *Amphipopyrum muticum*, (Riley, R., et al., 1966) and several diploid and tetraploid *Agropyron* species have such a suppressor (Chen, Q., et al., 1993). Thus, this might necessitate ascertaining that transgenic wheat will not transfer genes to the indigenous wild/weedy relatives in each locality where transgenic wheat is to be cultivated. Clearly these two interrelated mechanisms can be effective in preventing stable transgene introgression to *Ae. cylindrica* in the parts of the world where it is the sole problem-weed known to stably introgress genes from wheat.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this

application shall not be construed as an admission that such reference is available as prior art to the present invention.

References

(Additional references are cited in text)

1. Jasieniuk, M. et al. Evaluation of models predicting winter wheat yield as a function of winter wheat and jointed goatgrass densities. *Weed Science* 49, 48-60
5 (2001).
2. Stone, A. E. & Peeper, T. F. Characterizing jointed goatgrass (*Aegilops cylindrica*) x winter wheat hybrids in Oklahoma. *Weed Science* 52, 742-745 (2004).
3. Kihara, H. Considerations on the evolution and distribution of *Aegilops* species based on the analyser-method. *Cytologia* 19, 336-342 (1954).
- 10 4. Kimber, G. & Tsunewaki, K. Genome symbols and plasma types in the wheat group. in 7th International Wheat Genetics Symposium 1209-1211 (Cambridge, 1988).
5. Gressel, J. *Molecular biology of weed control* (Taylor and Francis, London, 2002).
- 15 6. Wang, Z. N., Zemetra, R. S., Hansen, J. & Mallory-Smith, C. A. The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. *Weed Science* 49, 340-345 (2001).
7. Dhaliwal, H. S., Harjit, S. & William, M. Transfer of rust resistance from *Aegilops ovata* into bread wheat (*Triticum aestivum* L.) and molecular
20 characterization of resistant derivatives. *Euphytica* 126, 153-159 (2002).
8. Ganeva, G., Georgieva, V., Panayotova, M., Stoilova, Z. & Balevska, P. The transfer of genes for brown rust resistance from *Aegilops umbellulata* Eig, to *Triticum aestivum* L. genome. *Russian Journal of Genetics* 36, 60-65 (2000).
9. Martin-Sanchez, J. A. et al. A new Hessian fly resistance gene (H30)
25 transferred from the wild grass *Aegilops triuncialis* to hexaploid wheat. *Theoretical and Applied Genetics* 106, 1248-1255 (2003).
10. Spetsov, P., Mingeot, D., Jacquemin, J. M., Samardjieva, K. & Marinova, E. Transfer of powdery mildew resistance from *Aegilops variabilis* into bread wheat. *Euphytica* 93, 49-54 (1997).
- 30 11. Sharma, H. et al. Introgression and characterization of barley yellow dwarf virus-resistance from *Thinopyrum intermedium* into wheat. *Genome* 38, 406-413 (1995).
12. Guadagnuolo, R., Savova-Bianchi, D. & Felber, F. Gene flow from wheat (*Triticum aestivum* L.) to jointed goatgrass (*Aegilops cylindrica* Host), as revealed by

RAPD and microsatellite markers. Theoretical and Applied Genetics 103, 1-8 (2001a).

13. Hegde, S. G. & Waines, J. G. Hybridization and introgression between bread wheat and wild and weedy relatives in north America. Crop Science 44, 1145-1155 (2004).

14. Morrison, L. A., Cremieux, L. C. & Mallory-Smith, C. A. Infestations of jointed goatgrass (*Aegilops cylindrica*) and its hybrids with wheat in Oregon wheat fields. Weed Science 50, 737-747 (2002).

15. Morrison, L. A., Riera-Lizarazu, O., Cremieux, L. & Mallory-Smith, C. A. Jointed goatgrass (*Aegilops cylindrica* Host) x wheat (*Triticum aestivum* L.) hybrids: Hybridization dynamics in Oregon wheat fields. Crop Science 42, 1863-1872 (2002).

16. Wang, Z. N. et al. Visualization of A and B genome chromosomes in wheat (*Triticum aestivum* L.) x jointed goatgrass (*Aegilops cylindrica* Host) backcross progenies. Genome 43, 1038-1044 (2000).

17. Wang, Z. N. et al. Determination of the paternity of wheat (*Triticum aestivum* L.) x jointed goatgrass (*Aegilops cylindrica* Host) BC1 plants by using genomic in situ hybridization (GISH) technique. Crop Science 42, 939-943 (2002).

18. Zemetra, R. S., Hansen, J. & Mallory-Smith, C. A. Potential gene transfer between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*). Weed Science 46, 313-317 (1998).

19. Weissmann, S., Feldman, M. & Gressel, J. Sequence evidence for sporadic inter-generic DNA introgression from wheat into a wild *Aegilops* species. Molecular Biology and Evolution (2005) (in press at time of patent submission).

20. Guadagnuolo, R., Savova-Bianchi, D., Keller-Senften, J. & Felber, F. Search for evidence of introgression of wheat (*Triticum aestivum* L.) traits into sea barley (*Hordeum marinum* s.str. Huds.) and bearded wheatgrass (*Elymus caninus* L.) in central and northern Europe, using isozymes, RAPD and microsatellite markers. Theoretical and Applied Genetics 103, 191-196 (2001b).

21. Lyon, D. J., Bussan, A. J., Evans, J. O., Mallory-Smith, C. A. & Peeper, T. F. Pest management implications of glyphosate-resistant wheat (*Triticum aestivum*) in the western United States. Weed Technology, 680-690 (2002).

22. Gressel, J. Multiple resistances to wheat selective herbicides: New challenges to molecular biology. Oxford Surveys of Plant Molecular and Cellular Biology 5, 195-203 (1988).

23. De Prado, J. L., Osuna, M. D., Heredia, A. & De Prado, R. *Lolium rigidum*, a pool of resistance mechanisms to ACCase inhibitor herbicides. *Journal of Agriculture Food and Chemistry* 53, 2185-2191 (2005).
24. Neve, P., Sadler, J. & Powles, S. B. Multiple herbicide resistance in a
5 glyphosate-resistant rigid ryegrass (*Lolium rigidum*) population. *Weed Science* 52, 920-928 (2004).
25. Yu, Q., Cairns, A. & Powles, S. B. Paraquat resistance in a population of *Lolium rigidum*. *Functional Plant Biology* 31, 247-254 (2004).
26. Price, L. J., Herbert, D., Moss, S. R., Cole, D. J. & Harwood, J. L.
10 Graminicide insensitivity correlates with herbicide-binding co-operativity on acetyl-CoA carboxylase isoforms. *Biochemistry Journal* 375, 415-423 (2003).
27. Cocker, K. M., Moss, S. R. & Coleman, J. O. D. Multiple mechanisms of resistance to fenoxaprop-P-ethyl in United Kingdom and other European populations of herbicide-resistant *Alopecurus myosuroides* (black-grass). *Pesticide Biochemistry and Physiology* 65, 169-180 (1999).
15
28. Beckie, H. J. et al. Nature, occurrence, and cost of herbicide-resistant wild oat (*Avena fatua*) in small-grain production areas. *Weed Technology* 13, 612-625 (1999).
29. Mengistu, L. W., Messersmith, C. G. & Christoffers, M. J. Diversity of herbicide resistance among wild oat sampled 36 yr apart. *Weed Science* 51, 764-773
20 (2003).
30. Kern, A. J. et al. Two recessive gene inheritance for triallate resistance in *Avena fatua* L. *Journal of Heredity* 93, 48-50 (2002).
31. Delye, C., Menchari, Y., Michel, S. & Darmency, H. Molecular bases for sensitivity to tubulin-binding herbicides in green foxtail. *Plant Physiology* 136, 3920-
25 3932 (2004).
32. De Prado, R., Osuna, M. D. & Fischer, A. J. Resistance to ACCase inhibitor herbicides in a green foxtail (*Setaria viridis*) biotype in Europe. *Weed Science* 53, 506-512 (2004).
33. Volenberg, D. S., Stoltenberg, D. E. & Boerboom, C. M. Green foxtail
30 (*Setaria viridis*) resistance to acetolactate synthase inhibitors. *Phytoprotection* 83, 99-109 (2002).
34. De Prado, R., Lopez-Martinez, N. & Gonzalez-Gutierrez, J. Identification of two mechanisms of atrazine resistance in *Setaria faberi* and *Setaria viridis* biotypes. *Pesticide Biochemistry and Physiology* 67, 114-124 (2000).

35. Preston, C. Herbicide resistance in weeds endowed by enhanced detoxification: complications for management. *Weed Science* 52, 448-453 (2004).
36. Yenish, J. R. & Young, F. L. Winter wheat competition against jointed goatgrass (*Aegilops cylindrica*) as influenced by wheat plant height, seeding rate, and seed size. *Weed Science* 52, 996-1001 (2004).
37. Ball, D. A., Young, F. L. & Ogg, A. G. Selective control of jointed goatgrass (*Aegilops cylindrica*) with imazamox in herbicide-resistant wheat. *Weed Technology* 13, 77-82 (1999).
38. Anderson, J. A., Matthiesen, L. & Hegstad, J. Resistance to an imidazolinone herbicide is conferred by a gene on chromosome 6DL in the wheat line cv. 9804. *Weed Science* 52, 83-90 (2004).
39. Kroiss, L. J. et al. Marker-assessed retention of wheat chromatin in wheat (*Triticum aestivum*) by jointed goatgrass (*Aegilops cylindrica*) backcross derivatives. *Crop Science* 44, 1429-1433 (2004).
40. Ellstrand, N. C. *Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives* (John Hopkins University Press, Baltimore, 2003).
41. Stewart, C. N., Halfhill, M. D. & Warwick, S. I. Transgene introgression from genetically modified crops to their wild relatives. *Nature Reviews: Genetics* 4, 806-817 (2003).
42. Snyder, J. R., Mallory-Smith, C. A., Balter, S., Hansen, J. L. & Zemetra, R. Seed production on *Triticum aestivum* by *Aegilops cylindrica* hybrids in the field. *Weed Science*, 588-593 (2000).
43. Oliver, M. J., Luo, H., Kausch, A. & Collins, H. in *Proceedings of the International Symposium on the Biosafety of Genetically Modified Organisms* 154-161 (INRA, Montpellier, 2004).
44. Oliver, M. J., Quisenberry, J. E., Trolinder, N. L. G. & Keim, D. L. (US patent 5,723,765, 1998).
45. Daniell, H. Molecular strategies for gene containment in transgenic crops. *Nature Biotechnology* 20, 581-586 (2002).
46. Maliga, P. Plastid transformation in higher plants. *Annual Review of Plant Biology* 55, 289-313 (2004).
47. Wang, T. et al. Low frequency transmission of a plastid-encoded trait in *Setaria italica*. *Theoretical and Applied Genetics* 108, 315-320 (2004).

48. Darmency, H. in *Herbicide Resistance in Plants: Biology and Biochemistry* (eds. Powles, S. B. & Holtum, J. A. M.) 263-298 (Lewis, Boca-Raton, 1994).
49. Gressel, J. Tandem constructs: preventing the rise of superweeds. *Trends in Biotechnology* 17, 361-366 (1999).
- 5 50. Al-Ahmad, H., Dwyer, J., Moloney, M. & Gressel, J. Mitigation of establishment of *Brassica napus* transgenes in volunteers using a tandem construct containing a selectively unfit gene. *Plant Biotech. Journal* (2005a). In Press.
51. Al-Ahmad, H., Galili, S. & Gressel, J. Tandem constructs to mitigate transgene persistence: tobacco as a model. *Molecular Ecology* 13, 697-710 (2004).
- 10 52. Al-Ahmad, H., Galili, S. & Gressel, J. Poor competitive fitness of transgenically mitigated tobacco in competition with the wild type in a replacement series. *Planta* (2005b). In Press.
53. Al-Ahmad, H. & Gressel, J. Mitigation using a tandem construct containing a selectively unfit gene precludes establishment of *Brassica napus* transgenes in hybrids and backcrosses with weedy *Brassica rapa*. *Plant Biotech. Journal* (2005b). In Press .
- 15 54. Okamoto, M. Asynaptic effect of chromosome V. *Wheat Information Service* 5, 6-8 (1957).
55. Riley, R. & Chapman, V. Genetic control of the cytologically diploid behavior of hexaploid wheat. *Nature* 182, 713-715 (1958).
- 20 56. Sears, E. R. & Okamoto, M. Intergenomic chromosome relationships in hexaploid wheat. in *Proceedings of the 10th International Congress of Genetics* 258-259 (University of Toronto, Toronto, 1958).
57. Sears, E. R. Genetic control of chromosome pairing in wheat. *Annual Review of Genetics* 10, 31-51 (1976).
- 25 58.
60. Jenczewski, E. et al. PrBn, a major gene controlling homoeologous pairing in oilseed rape (*Brassica napus*) haploids. *Genetics* 164, 645-653 (2003).
61. Udall, J. A., Quijada, P. A. & Osborn, T. C. Detection of chromosomal rearrangements derived from homoeologous recombination in four mapping
- 30 populations of *Brassica napus* L. *Genetics* 169, 967-979 (2005).
62. Morris, R. & Sears, E. R. in *Wheat and wheat improvement* (ed. Quisenberry, K. S.) 19-88 (American Society of Agronomy Inc., Madison, Wisconsin, 1967).

63. Jampates, R. & Dvorak, J. Location of the *Ph1* locus in the metaphase chromosome map and the linkage map of the 5Bq arm of wheat. *Canadian Journal of Genetics and Cytology* 28, 511-519 (1986).
64. Sears, E. R. Mutations in wheat that raise the level of meiotic chromosome pairing. in 16th Stadler Genetics Symposium pp. 295-300 (Plenum Press, New York, 1984).
65. Puchta, H. Gene replacement by homologous recombination in plants. *Plant Molecular Biology* 48, 173-182 (2002).
66. Reiss, B. Homologous recombination and gene targeting in plant cells. 10 *International Review of Cytology* 228, 85-139 (2003).
67. Li, H. Q. & Li, M. R. RecQ helicase enhances homologous recombination in plants. *FEBS letters* 574, 151-155 (2004).
68. Mariani, C. et al. A chimeric ribonuclease-inhibitor gene restores fertility to male sterile plants. *Nature* 357, 384-387 (1992).
- 15 69. Logemann, J., Jach, G., Tommerup, H., Mundy, J. & Schell, J. Expression of a barley ribosome-inactivating protein leads to increased fungal protection in transgenic tobacco plants. *Bio-Technology* 10, 305-308 (1992).
70. Jach, G. et al. Enhanced quantitative resistance against fungal disease by combinatorial expression of different barley antifungal proteins in transgenic tobacco. 20 *Plant Journal* 8, 97-109 (1995).
71. Korchuganov, D. S. et al. 187E mutation prevents barstar dimerization. *Russian Journal of Bioorganic Chemistry* 30, 577-581 (2004).
72. Hartley, R. W. Barnase and barstar - two small proteins to fold and fit together. *Trends in Biochemical Sciences* 14, 450-454 (1989).
- 25 73. Riley, R., Campbell, C. S., Young, R. M. & Belfield, A. M. Control of meiotic chromosome pairing by the chromosomes of homoeologous group 5 of *Triticum aestivum*. *Nature* 212, 1475-1477 (1966).
74. Chen, Q., Jahier, J. & Cauderon, Y. The B-chromosome system of inner Mongolian *Agropyron* Gaertn .3. Cytogenetical evidence for B-A pairing at 30 metaphase-I. *Hereditas* 119, 53-58 (1993).

WHAT IS CLAIMED IS:

1. A method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant thereby preventing the stable transgene introgression into the related, non-cultivated plant.
2. A transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant.
3. A method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a *Ph1* locus of the transgenic cultivated plant thereby preventing the transgene flow into the related, non-cultivated plant.
4. The method or the transgenic cultivated plant of any of claims 1, 2 and 3, wherein said exogenous polynucleotide comprises a nucleic acid sequence encoding a polynucleotide or a polypeptide product.
5. The method or the transgenic cultivated plant of any of claims 1, 2 and 3, wherein said genetic locus being in linkage disequilibrium with said *Ph1* locus is set forth by SEQ ID NO:13, 14, 15, 16, 17 and 39 .
6. The method or the transgenic cultivated plant of any of claims 1, 2 and 3, wherein the transgenic cultivated plant is wheat.

7. The method or the transgenic cultivated plant of claim 6, wherein said wheat is *Triticum aestivum* ssp. *aestivum* and *Triticum turgidum* ssp. *durum*.

8. The method or the transgenic cultivated plant of any of claims 1, 2 and 3, wherein said related, non-cultivated plant is selected from the group consisting of *Aegilops* spp., *Hordeum* spp., *Elymus* spp., *Agropyron* spp., *Eremopyrum* ssp., *Secale* spp., *Dasypyrum* spp., *Heteranthelium* ssp., *Amblyopyrum* spp., *Henrardia* spp.,
5 *Thinopyrum* spp., *Leymus* spp., *Psathyrostachys* spp., *Hystrix* spp., *Hordelymus* ssp., *Taeniatherum* ssp. and *Crithopsis* ssp.

9. The method or the transgenic cultivated plant of any of claims 1, 2 and 3, wherein said exogenous polynucleotide comprises a nucleic acid sequence encoding a suppressor of a gene or gene product lethal to the related, non-cultivated plant.

10. The method of claim 9, further comprising inserting a second exogenous polynucleotide into a second genetic locus of the transgenic cultivated plant, said second genetic locus being in random association with said *Ph1* locus of the transgenic cultivated plant.

11. The transgenic cultivated plant of claim 9, further comprising a second exogenous polynucleotide positioned in a second genetic locus of the transgenic cultivated plant, said second genetic locus being in random association with said *Ph1* locus of the transgenic cultivated plant.

12. The method or the transgenic cultivated plant of any of claims 10 and 11, wherein said second exogenous polynucleotide comprises a nucleic acid sequence encoding a product lethal to the related, non-cultivated plant.

13. The method or the transgenic cultivated plant claim 12, wherein said second exogenous polynucleotide further comprises a second nucleic acid sequence encoding a transgene.

14. The method or the transgenic cultivated plant of any of claims 4 and 13, wherein said transgene is a polynucleotide or a polypeptide.

15. The method or the transgenic cultivated plant of any of claims 4 and 13, wherein said polynucleotide or polypeptide product endows the transgenic cultivated plant with a commercially desirable trait selected from the group consisting of herbicide resistance, disease resistance, insect resistance and nematode resistance, environmental stress resistance, high productivity, modified agronomic quality, enhanced yield, modified ripening, and bioremediation.

16. The method or the transgenic cultivated plant of any of claims 4 and 13, wherein said exogenous polynucleotide further comprises a promoter for directing an expression of said nucleic acid sequence in the transgenic cultivated crop.

17. The method or the transgenic cultivated plant of claim 16, wherein said promoter is selected from the group consisting of FMV, 35S, E8, E4, E17, J49, 2A11, and Tap1.

18. The method or the transgenic cultivated plant of claim 12, wherein said product lethal to the related, non-cultivated plant is barnase.

19. The method or the transgenic cultivated plant of claim 9, wherein said suppressor of said product lethal to the related, non-cultivated plant is *barstar*.

20. A method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a centromeric region of a chromosome of the transgenic cultivated plant thereby preventing the transgene flow into the related, non-cultivated plant.

21. A transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a centromeric region of a chromosome of the transgenic cultivated plant.

22. A method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a centromeric region of a chromosome of the transgenic cultivated plant.

23. The method or the transgenic cultivated plant of any of claims 20, 21 and 22, wherein said exogenous polynucleotide comprises a nucleic acid sequence encoding a polynucleotide or a polypeptide product.

24. The method or the transgenic cultivated plant of any of claims 20, 21 and 22, wherein the transgenic cultivated plant is wheat.

25. The method or the transgenic cultivated plant of claim 24, wherein said wheat is *Triticum aestivum* ssp. *aestivum* and/or *Triticum turgidum* ssp. *durum*.

26. The method or the transgenic cultivated plant of any of claims 20, 21 and 22, wherein said related, non-cultivated plant is selected from the group consisting of *Aegilops* spp., *Hordeum* spp., *Elymus* spp., *Agropyron* spp., *Eremopyrum* spp., *Secale* spp., *Dasypyrum* spp., *Heteranthelium* spp., *Amblyopyrum* spp., *Henrardia* spp., *Thinopyrum* spp., *Leymus* spp., *Psathyrostachys* spp., *Hystrix* spp., *Hordelymus* spp., *Taeniatherum* spp., and *Crithopsis* spp.

27. The method or the transgenic cultivated plant of claim 23, wherein said polynucleotide or polypeptide product endows the transgenic cultivated plant with a commercially desirable trait selected from the group consisting of herbicide resistance, disease resistance, insect resistance and nematode resistance, environmental stress resistance, high productivity, modified agronomic quality, enhanced yield, modified ripening, and bioremediation.

28. The method or the transgenic cultivated plant of claim 23, wherein said exogenous polynucleotide further comprises a promoter for directing an expression of said nucleic acid sequence in the transgenic cultivated crop.

29. The method or the transgenic cultivated plant of claim 28, wherein said promoter is selected from the group consisting of FMV, 35S, E8, E4, E17, J49, 2A11, and Tap1.

30. The method or the transgenic cultivated plant of any of claims 20, 21 and 22, wherein said centromeric region of the transgenic cultivated plant is on chromosome 5 of sub genome B of *Triticum aestivum* ssp. *aestivum* and/or *Triticum turgidum* ssp. *durum*.

31. The method or the transgenic cultivated plant of any of claims 20, 21 and 22, wherein said centromeric region of the transgenic cultivated plant is a sub-centromeric region of the transgenic cultivated plant.

32. A method of generating a transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the method comprising inserting an exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a locus including a gene capable of preventing homoeologous recombination, thereby preventing the stable introgression into the related, non-cultivated plant.

33. A transgenic cultivated plant characterized by reduced stable transgene introgression to a related, non-cultivated plant, the transgenic cultivated plant comprising an exogenous polynucleotide positioned in a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a locus including a gene capable of preventing homoeologous recombination.

34. A method of reducing introgression of an exogenous polynucleotide of a transgenic cultivated plant into a related, non-cultivated plant, the method comprising inserting the exogenous polynucleotide into a genetic locus of the transgenic cultivated plant, said genetic locus being in linkage disequilibrium with a locus including a gene capable of preventing homoeologous recombination thereby reducing introgression of the exogenous polynucleotide into the related, non-cultivated plant.

35. The method or the transgenic cultivated plant of any of claims 32, 33 and 34, wherein said exogenous polynucleotide comprises a nucleic acid sequence encoding a polynucleotide or a polypeptide product.

36. The method or the transgenic cultivated plant of any of claims 32, 33 and 34, wherein said related, non-cultivated plant is selected from the group consisting of *Aegilops* spp., *Hordeum* spp., *Elymus* spp., *Agropyron* spp., *Eremopyrum* spp., *Secale* spp., *Dasypyrum* spp., *Heteranthelium* spp., *Amblyopyrum* spp., *Henrardia* spp., *Thinopyrum* spp., *Leymus* spp., *Psathyrostachys* spp., *Hystrix* spp., *Hordelymus* spp., *Taeniatherum* spp., and *Crithopsis* spp.

37. The method or the transgenic cultivated plant of any of claims 32, 33 and 34, wherein said exogenous polynucleotide endows the transgenic cultivated plant with a commercially desirable trait selected from the group consisting of herbicide resistance, disease resistance, insect resistance and nematode resistance, environmental stress resistance, high productivity, modified agronomic quality, enhanced yield, modified ripening, and bioremediation.

1/7

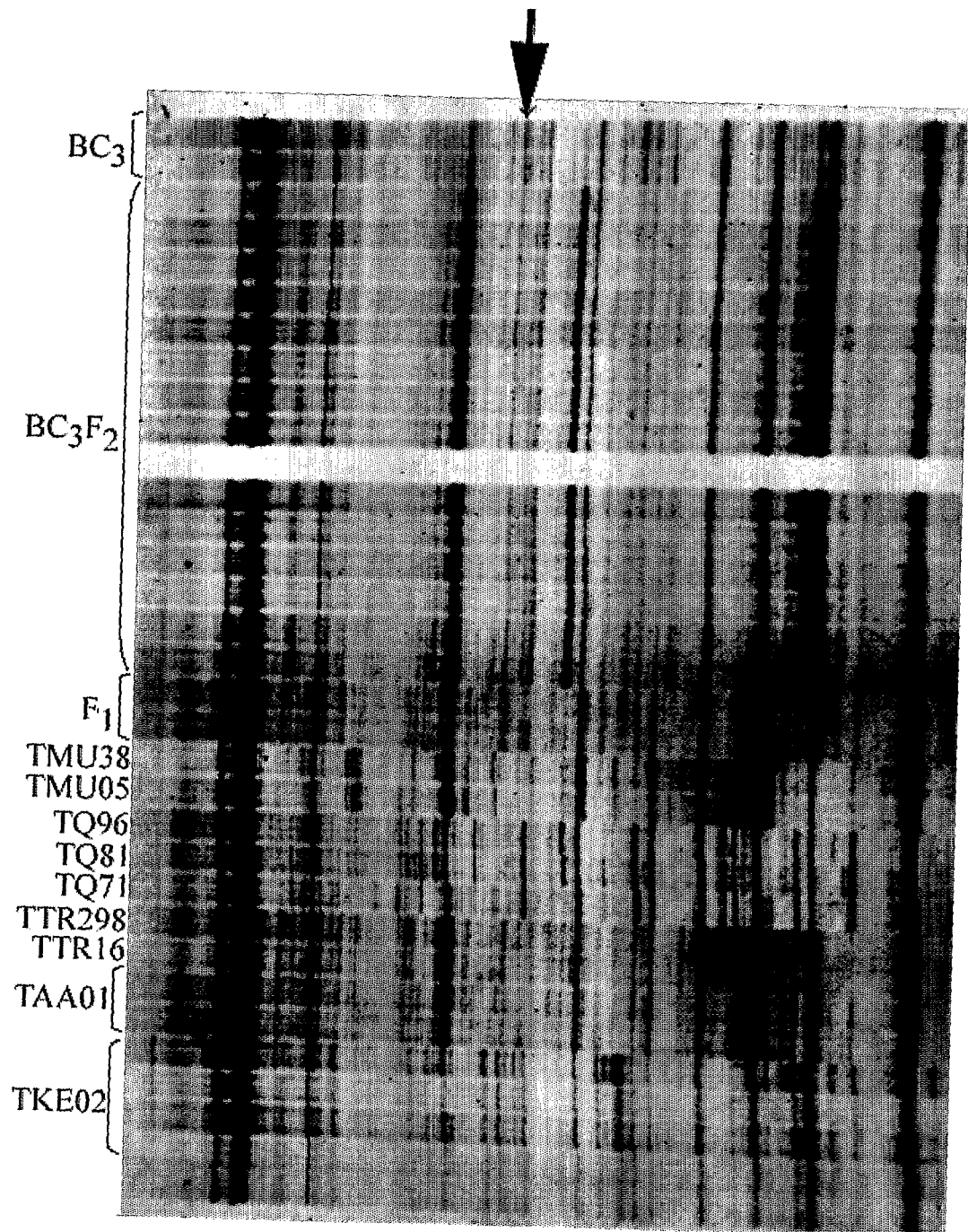


Fig. 1

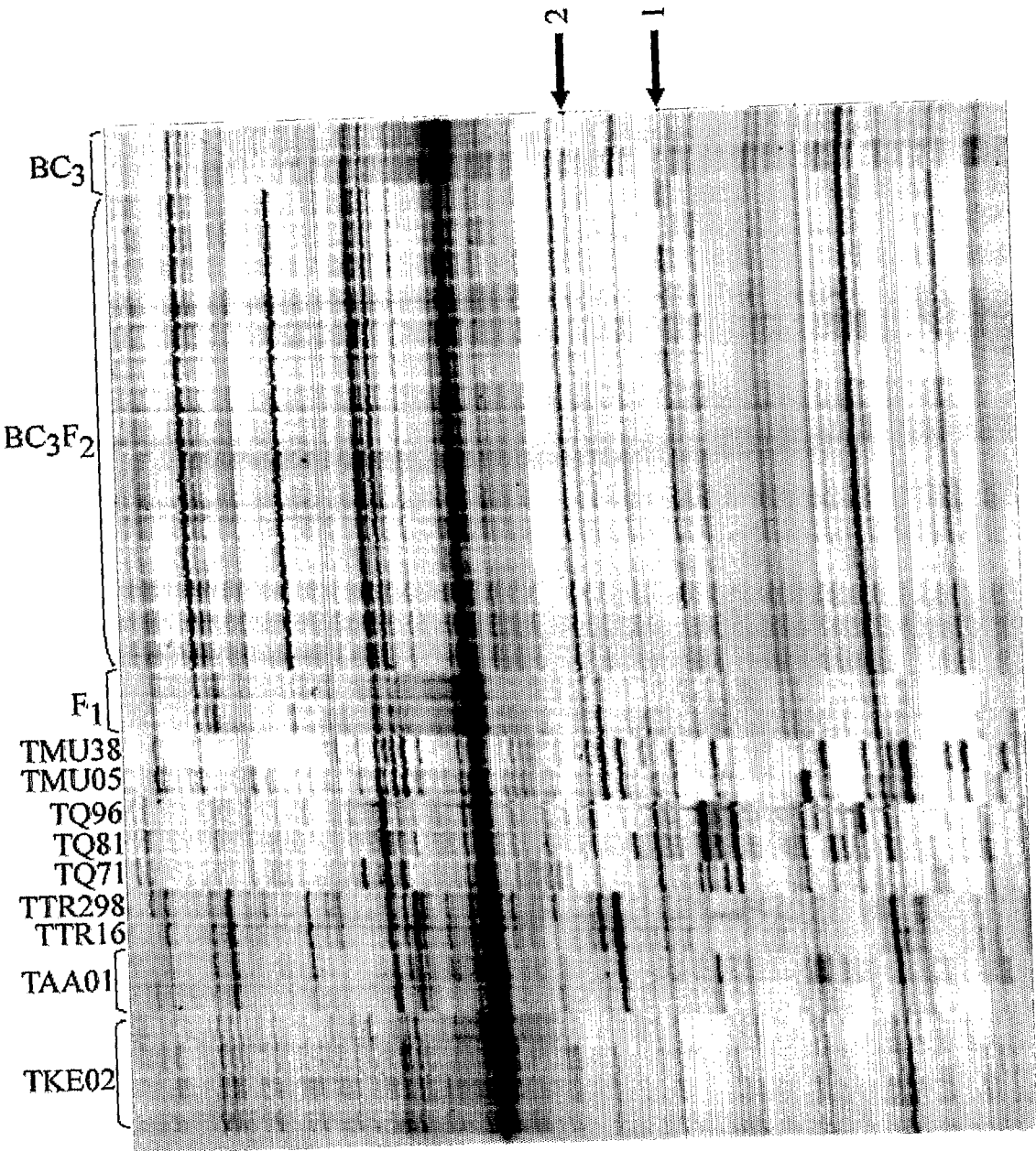


Fig. 2

3/7

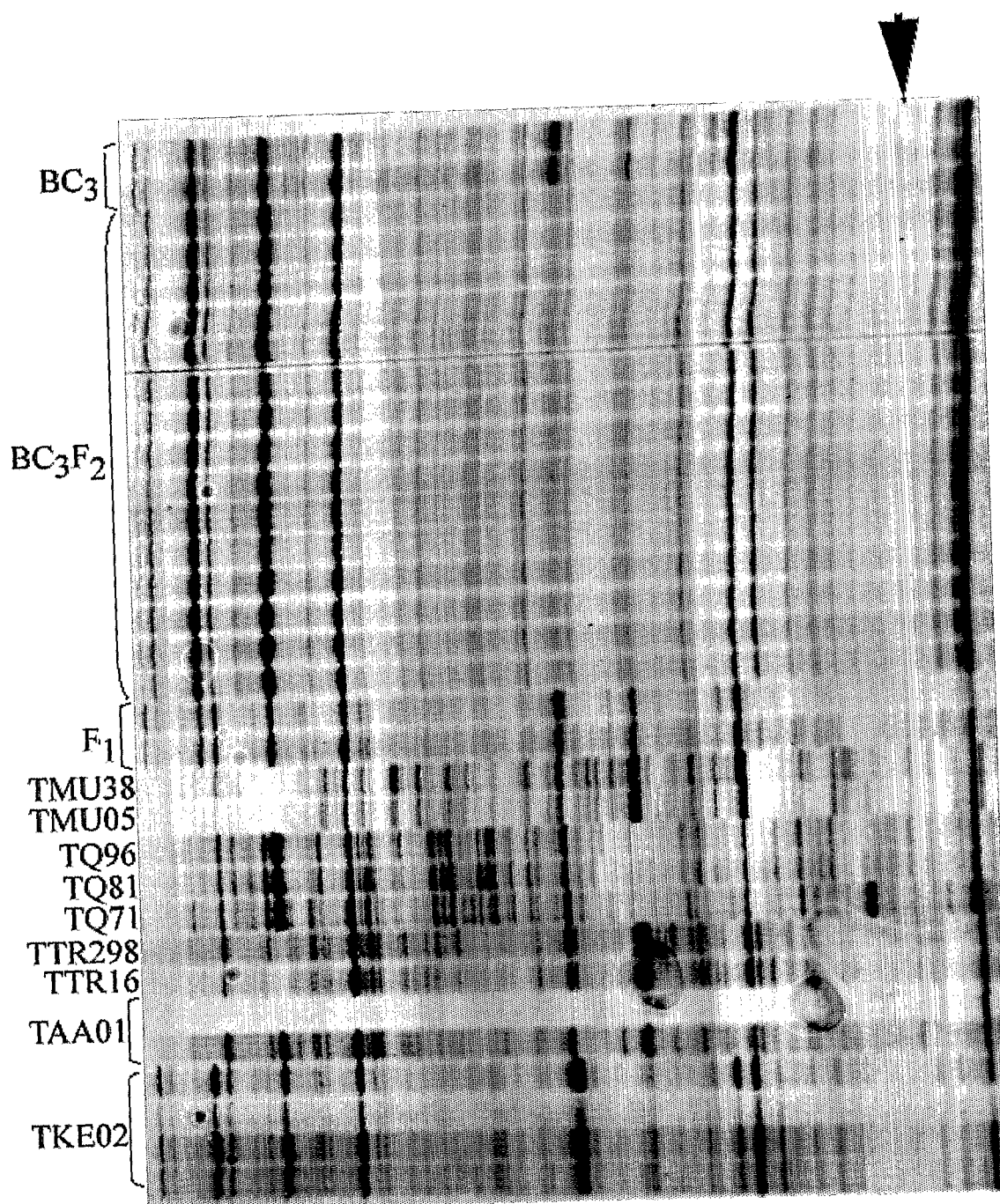


Fig. 3

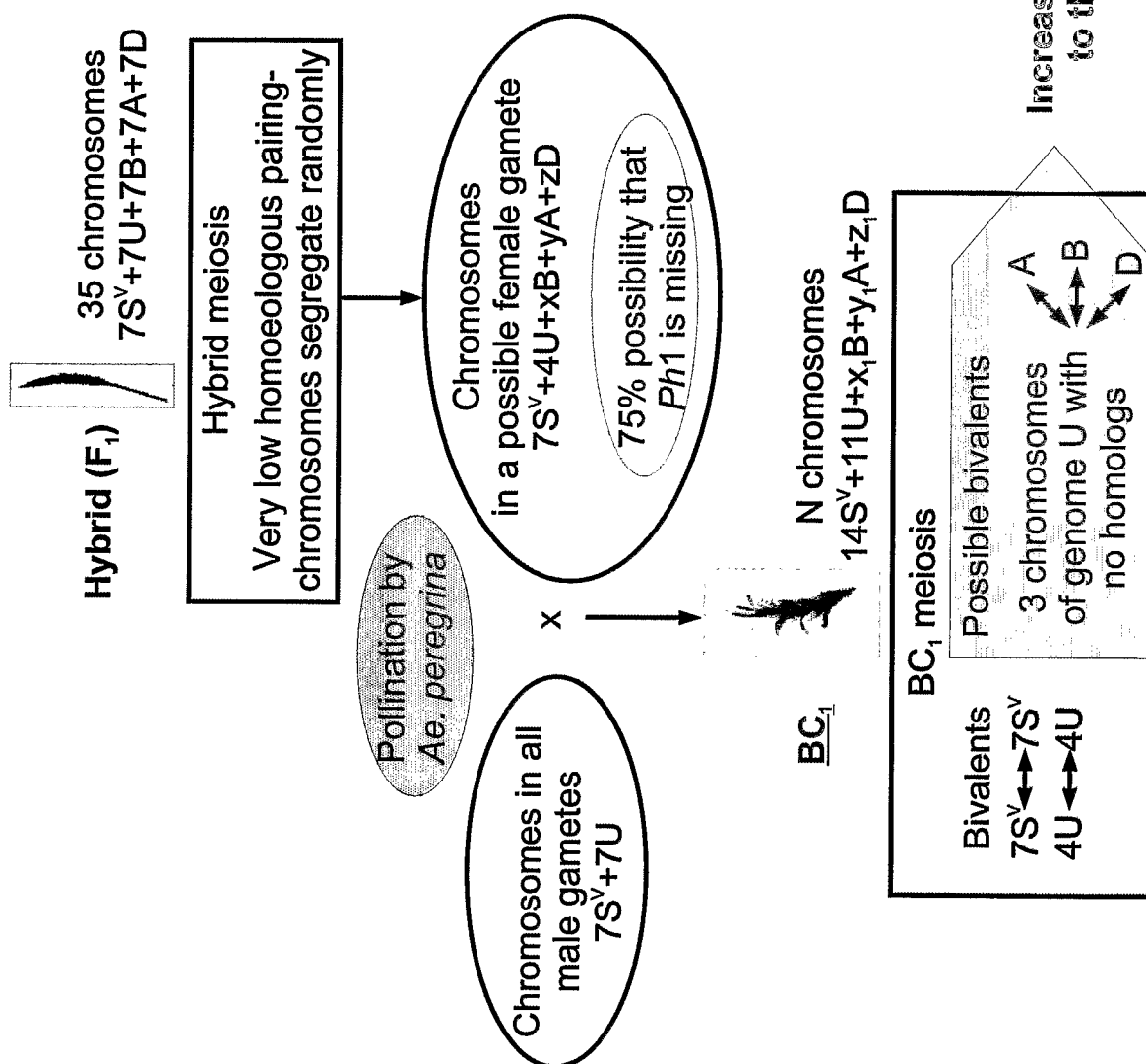
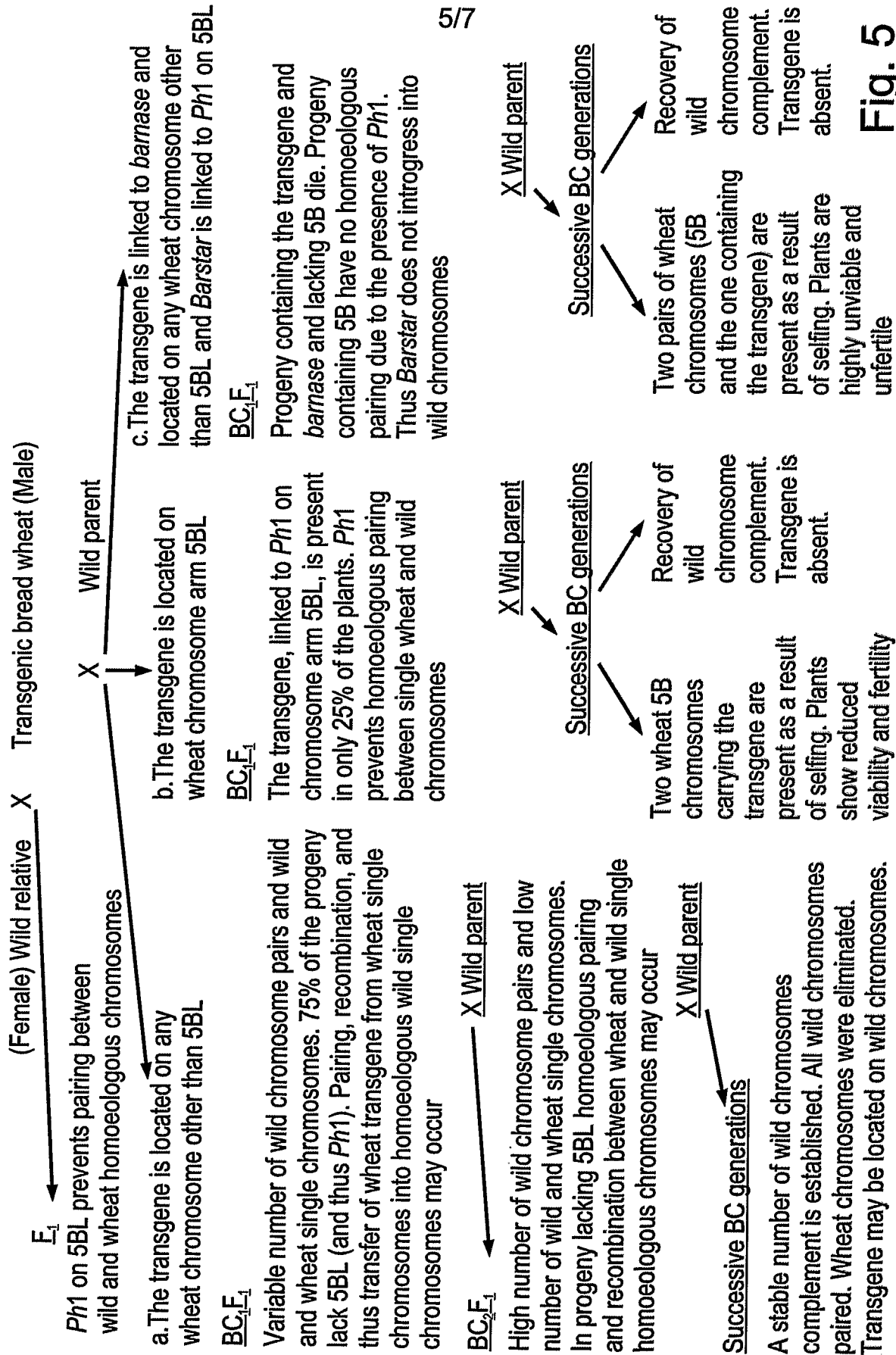


Fig. 4



5/7

Fig. 5

6/7

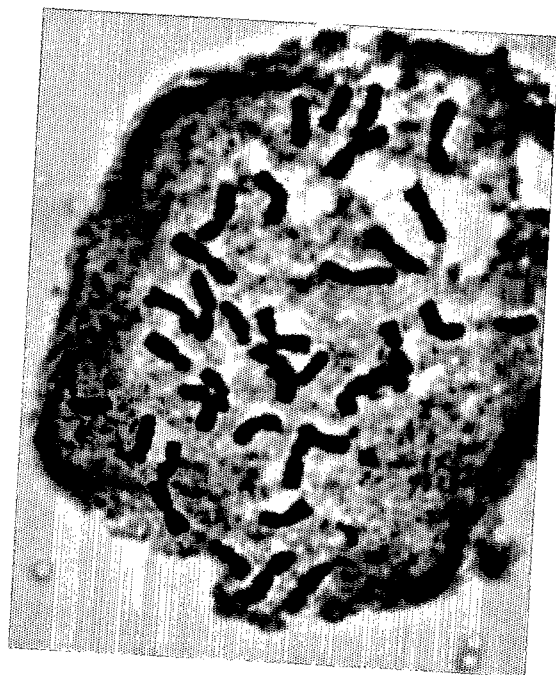


Fig. 6



Fig. 7a

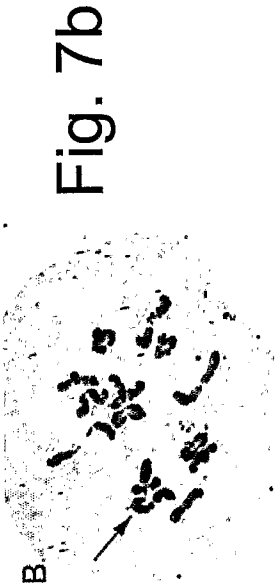


Fig. 7b

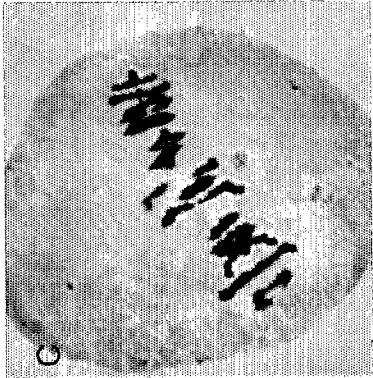


Fig. 7c



Fig. 7d

SEQUENCE LISTING

<110> Weissmann, Sarit
Feldman, Moshe
Gressel, Jonathan

<120> METHODS OF PREVENTING GENE FLOW IN PLANTS

<130> 32236

<160> 46

<170> PatentIn version 3.3

<210> 1
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 1
gatgagtcct gagtaactg 19

<210> 2
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 2
gatgagtcct gagtaactt 19

<210> 3
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 3
gatgagtcct gagtaacag 19

<210> 4
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 4
gatgagtcct gagtaacat 19

<210> 5
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 5
gatgagtcct gagtaacta 19

<210> 6
<211> 19

<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 6
gatgagtcct gagtaactc 19

<210> 7
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<400> 7
gatgagtcct gagtaacag 19

<210> 8
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<220>
<221> misc_feature
<223> FAM (6-carboxyfluorescein) labeled oligonucleotide

<400> 8
gactgcgtac caattcact 19

<210> 9
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<220>
<221> misc_feature
<223> TET (4, 7, 2', 7'-tetrachloro-6-carboxyfluorescein) labeled oligonucleotide

<400> 9
gactgcgtac caattcaca 19

<210> 10
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Single strand DNA oligonucleotide

<220>
<221> misc_feature
<223> HEX (4, 7, 2', 4', 5', 7'-hexachloro-6-carboxyfluorescein) labeled oligonucleotide

<400> 10
gactgcgtac caattcaac 19

<210> 11
<211> 19

<212> DNA
 <213> Artificial sequence

 <220>
 <223> Single strand DNA oligonucleotide

 <220>
 <221> misc_feature
 <223> FAM (6-carboxyfluorescein) labeled oligonucleotide

 <400> 11
 gactgcgtac caattcacc 19

 <210> 12
 <211> 19
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Single strand DNA oligonucleotide

 <220>
 <221> misc_feature
 <223> HEX (4, 7, 2', 4', 5', 7'-hexachloro-6-carboxyfluorescein) labeled oligonucleotide

 <400> 12
 gactgcgtac caattcacg 19

 <210> 13
 <211> 306
 <212> DNA
 <213> Triticum aestivum

 <400> 13
 cttgccctt caggcgagag agagagagag agagagagag atagatagat agatagatag 60
 atagatagag agagagagag agagacagac tcataggtgg ggccggctgt taggtttaag 120
 agagggagag agagaaacag aggtgggtgg ctctgtcact ggtggttctc ccgcgaggag 180
 accacggcaa ccgccggaga ggaggggggtg gatcaggcgc attaaggcaa gcacggggt 240
 accctacatg acggctaagt tggacgaaca acgtcaaat ggtcatctcc agagaaggcg 300
 ggcggc 306

 <210> 14
 <211> 677
 <212> DNA
 <213> Triticum aestivum

 <400> 14
 attcatggaa aaaaatgatt ttgatgaaat tttttgaag attgatgaac aaatctcgca 60
 tttgatgaac attttttaat tcgattaaca attttcaatg aaaattttta aatcaacaaa 120
 aattaaactt aatgaacatt ttttgaattt gatgaacaaa aaaaggctcct ctgtttgaaa 180
 taaagtgtg aaaactgaaa atgtacacaa ttttataaga tgttcacaaa attggtaaaa 240
 aaaaatactc atgaacttaa aatgttcaca atttagaaaa aataatcatg tattttaaaa 300
 atggtcatga ttgcaaaggc tttcacaaat ttcaataaat gttcatgaat ctgaaagatg 360
 cttgcaaatt taaaaagaaa aagaaaaatt aaacaaaatc gaaattaaaa atggaaataa 420
 taataataat aataataata ataataataa taataataat aataataata ataataataa 480
 acagaaagga aaaagaaaag gagaaagaaa gaaaaaacag aaaggaaaga tacctgttga 540
 gagaagggtc ttgaaccttc ccaaaaccga aggggaagaa atctgttaat aggccagctg 600

aaaggaggat cgaaggcagg tggggagtag gcgctaagta gataggttgt gccctatgcg 660
 caaatagaaa acctccc 677

<210> 15
 <211> 337
 <212> DNA
 <213> Triticum aestivum

<400> 15
 ctcaatcatc atctagacag cagacaaact aacggactga aacactgaca gcaggaaggc 60
 agatcatttc ttcttcttgg ccggcggtg gtcctcatct tcctcgtcct cctcatcctc 120
 gtcctcatca tcatcgtcgt catcctcgcc atcttcacca tcatcatcat cgtcatcacc 180
 atcatcatcg tcgctccccg caccagcgtc gtcgccatc gcctcagggt cgtcgtcctc 240
 gtcatcgtcc tcgccttctt cacctgagaa gtcttcccca gcatcggcac catcatcatc 300
 agcatcacca gtttcatcat cctcatcttc atcatcg 337

<210> 16
 <211> 316
 <212> DNA
 <213> Triticum aestivum

<400> 16
 acatacgcaa caggcttgag gaggcctaga gactcaaagc tgcaggggca gccggctatg 60
 atgatgatga tgatgatgat gatgatcggg gcgacgggaa ttcctccgat gaggaatgtg 120
 ttgatgaaga gattgctgcg cttgaggcgt taggggccgc ggctgccgga gcagaggttg 180
 ttgggcctca gatggccgaa acgccaagg aggaggaagg acttgagggt ggccctcagg 240
 ctctgggca atggcacgc ctttgcaaaa gaggtaaccg agaaccggtg gtttcttctt 300
 cggctcgggc aggccc 316

<210> 17
 <211> 390
 <212> DNA
 <213> Triticum aestivum

<400> 17
 ctgggtgctga gctcttggga gcaggagaac ttgtggatgg agtcgcttgg gacgacgtcg 60
 gcgaaggcgg gctactgccc ggaggaatca gtgcggtcct gctcttcttc ttggccactc 120
 gcttgggttg cttcgaacag gatacccat acctcgtggt gactccaaa gggcgcgga 180
 ccagcactac cggggcttca tcatcatcat catcatcatc atcatcatca gaaagtcgc 240
 agaggatctc cctctgacgc ggggcagagg attcctccac catcttgtct tcggtggcct 300
 cggaggaaca atcatcagag gacaggtgca ccaaagcgtt aggcggcggg ggatgccccg 360
 accgtacaag cccaaactcg tcgaagacag 390

<210> 18
 <211> 443
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> misc_feature
 <222> (2)..(2)
 <223> n is a, c, g, or t
 <220>

<221> misc_feature
 <222> (7)..(8)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (12)..(12)
 <223> n is a, c, g, or t

<400> 18
 tntttcnnac cncctgctta ctctctcttag gccagcttag caaactaaac tctagcggct 60
 ttgagagaga aattaggctc cccggctagt gaccacacac tcatgtcgaa aaaaggagtg 120
 ataaataata ataataataa taataataat aaaactttgc aatgactaag aagaagaaca 180
 cttattataa aacggctcaa ataaatttaa gagccctcaa gtgacttggg taaaagaaca 240
 gagtgtccct agtatgcctc tacttgacta gctcgtaat gaaagatgct taagtttcct 300
 agccatggac atgtgttgctc atttcatgaa cgagatcaca ttattagaga atgatgtgat 360
 ggaccagacc catccattag cttagcaciaa tgatcgttta gtttattgct attgctttct 420
 tcatgactta tacatgttcc tat 443

<210> 19
 <211> 532
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> n is a, c, g, or t

<400> 19
 cctaactatt tgancagtgg ataaaataat gttttaaatg cctttttact tgataaaaat 60
 actaaagtaa attatttaac tactacactt aatgtgacag taacctaaat tataatggta 120
 tttcagggtg agttgtttta ttttataaaa caaatttggg tataaaggat attagacaaa 180
 cagatgcttt aaaagaaaaa aaaacaaaaa ggtaaaagta aaggaaagag agagagagag 240
 agggagagag agagagagag agagagagag agagagagag aacaccacca ctggccgctg 300
 ggccttagcc caccacagtg agccggctca ccttttcccc gtgtggttga tccccttcct 360
 tactgctcct ccgaccgagg cgcacccccg ccagacaggg gcgcgtctcc ccgtactgac 420
 acatcgctgg cagggggata agggcagccc ctacggcctc gggctccctc cctactcacc 480
 cactcctctc tccctatcgc tctccctctc cgttctcgct tgctctgccc ct 532

<210> 20
 <211> 469
 <212> DNA
 <213> Triticum aestivum

<400> 20
 actcgtggcc ttgcagcgtc gagtcgcgcc aaaggtcaag gtcccggcaa tcgttgcaat 60
 agctgccaac atcactctat ttgtaagaga cagggcaacg ctaacataac aacatcaagg 120
 ggaaagaaaa acggttcggt tcaaagcgtg caatacctgc aaatcacgtt cgggaggatg 180
 aatgaagcac agggatcttg gaactgtgct tctggagcaa attcctttac gcgcaccaac 240
 ttttaacaat tctttcgcac tctctgtcat atatatattg gataatgaga atagtagtag 300
 taataataat aataataata ataataataa caaaggagtt aactctaaac caggaacacg 360
 acacagtatt catagacatg taataaataa ttcaaagtca ggaggcgata gtgctacatt 420

taagagatct atcaaaactaa aatagtctgt tatgcaccgg cgtacatgg 469

<210> 21
 <211> 461
 <212> DNA
 <213> Triticum aestivum

<400> 21
 ttgcaagga cagctaagaa cgtccatggt gcaactgcct atgaaacggc tatgcagcta 60
 atttacgtcg ttgtaaacga gtaccaacca aacacgtcaa agtattctca atacttcaaa 120
 atactttgat atgtaaaaac tgtggtatct agtacctata gtttaaaata ctgtagtttt 180
 ccaatactac agtttttgca ataccttggt gtcaaacaca gcctaagtta tgctataaca 240
 ctgaaattga ctgaccacaa ttttggttca gtcgattggt taggaattga ctgacttcct 300
 aaaatcgggc aaccgcttgt tgtcaaataa taataataat aataataata ataataataa 360
 taataataat aataataata ataataataa taataatagt aattaggga ctgccctata 420
 gccactccca tcattagaac tttggtatg attcatatag g 461

<210> 22
 <211> 609
 <212> DNA
 <213> Triticum aestivum

<400> 22
 gcgtgcaccg ggagaagaga cagcaggcct ccggaacccc gcctctcatg atcctgtacg 60
 ggagaggggc gatcagggtt ttggggagcg cactcgcgcg actgctggca gcgacgactt 120
 cgccaacgac gacttcttcc ccgacctcgg caacctcatc ttcgacgaca tgggcgacaa 180
 cgtcaacgcc ggcggtgctg ctcccgtac accgtatgtg attctatcct tcctattcga 240
 gatcgtggtg gaattcatgt ttctagtatg tgcctacat gtgatctatt catctgctat 300
 gctagtctcg atgattagtt taatctctac tactgtagtc atgatttacc ttctaattat 360
 tcggattaaa tctcgtagta acttgctcat atttccaaca tcaatttgtt catggagcta 420
 ctctctctct ctctctctct ctctctctct ctctctctct ctctctctct ctacagtgc 480
 ggagcttctt tagggccaac ccaggccatg ccccccccc ccaggttgtg tagttttggt 540
 ataccaatag cacataatta gacattagac atgaatgatg gagttctgcc caatctttga 600
 gtgaatggc 609

<210> 23
 <211> 454
 <212> DNA
 <213> Triticum aestivum

<400> 23
 ccctccctct atccctgaaa ccaataagaa tatcccttcg tttaattccc gcctacatgc 60
 accgtcggta gtactatctc tcggtgtctc tcaaacaac acgttatctc gatgtccact 120
 catgtcacat gcacacgcct tctctcccct gtttacgagc gtcttctctc tcgcaccccc 180
 tcgttgaggc cctctgccat aaacatcacc tctctatgat gaagcggcac acacttaatt 240
 actttccctg ccaatgactg catctctctc tctctctctc tctctctctc ttccttccct 300
 ctttcacatg atgttgaggc ccgagcctcg gtccataagt actttggata gctggcagcc 360
 cccgatgatg gggccgatga cgagcatgtt cctccctcga tgttcttgga gatctatttg 420
 atgtaatctt cttttttag tagtggttgg ggga 454

<210> 24
 <211> 471
 <212> DNA
 <213> Triticum aestivum

<400> 24
 cccccggttc cataacgccg atagtaattt ttataataat aataataata ataataataa 60
 taataataat aataataata ataataataa taataggata ccctatgatac ttgaagctaa 120
 tattaagaa tttattaatt cccaaaatat ttttaatgct atgatatggtg aaacttgaat 180
 aaagttgatg atatggctag gaacatggat agaattgctc atgatgcgga aaatctcata 240
 ttttaagattt tttaccaag cttgatatta atgagcccat taaagctttc tatgtgtcca 300
 ttaatgaaag taaagagaga atcgctagat tgagggctaa gcgagaattc ctgaaaaag 360
 cgactccgcc cgattttttac cgtaatcatg acaaagataa taaaatgatt ggtgtatcac 420
 tcatagaata tttattaagt aatataaaac ttgatgaaa aggtactgaa g 471

<210> 25
 <211> 380
 <212> DNA
 <213> Triticum aestivum

<400> 25
 ccttggcctg ccttccttct tctccaaac agctctagga gcgactctgt actgttacgc 60
 ataaaccaca cccggcagga ctaggggtgt tatctctcag gagagccccg aacctgggta 120
 tgtcttgctg ccgcgctcg ctcattgtga cctcgctctt ggagcccacc agtgccctcg 180
 agccccctcc tctcttttagc catcccttgg catctgccgt ggcccaccac gacaaccgcg 240
 gagttcttcg tgtttgctg tgctctctaa catagaaggg gagaagagag agagagagag 300
 agagagagag agagcagaca tggaaccac cagtaagtga aggcgaatag tacgtgctaa 360
 cgagccggtc ccaactaatgt 380

<210> 26
 <211> 566
 <212> DNA
 <213> Triticum aestivum

<220>
 <221> misc_feature
 <222> (7)..(10)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (14)..(15)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (18)..(18)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (32)..(32)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (57)..(57)
 <223> n is a, c, g, or t

```

<220>
<221> misc_feature
<222> (160)..(160)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (207)..(207)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (448)..(448)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (563)..(563)
<223> n is a, c, g, or t

<400> 26
agtgggnnnn taanntanat ctctttgaac anccggaact ggacactttc taggccnctc      60
agccaactta ttatgttcac taattattat tattattatt attattatta ttattattat      120
tatgattatt attattatta ttattattat tattatatgn ccgtgggcgg accaaaaaat      180
tggagaaatg cgccgtcctt tattagnagg tataaattta tcttcatcaa attgagcgca      240
tatatatctt ttccccaacc ttgtcaccaa cctccagacc actcctatga atgtgcctcc      300
cctcaagcat gcaccaaacg gtaccaaattg ttgatgcttc gccgcccggg cctctcctct      360
tctcctccct cgacctttcc tctctttctt gtgcaaggcg gccgcccggg gagttccgtc      420
cgccatagcc caccatccac gtcccgncg tcttagagga gcaccggcgt gcacaacctat      480
tgggttgccac ctctcaagg gcagccacaa atgtcagtaa agcttgcatg cctggaggcg      540
acttagagtc cccggggtac cngnct                                     566

<210> 27
<211> 541
<212> DNA
<213> Triticum aestivum

<400> 27
aatatattat aaaagttcag cgcaagtaca aagcgtagtc cggtgcatgt agcttccgct      60
tgcgagggtt ccagggaagg gtccgacctt ttgggtctt tagtacgcag ccttacccaa      120
catttctaca aaaggcagca gctttaccgc tgcgccaagg ctccccttcc agcgcaagta      180
caaagcattc caataataat aataataata ataataataa atcgagggtt ctggatcacc      240
aaacgatcac tatcgtcatt agaacaagat gccgacacgt tgctgttgcg cctcccatat      300
cggagctgac ctgacatgtc attgacaacc aagaagtctt catgcacacg cctctaagga      360
ctaatgctca gagttgcagt tgcgcccgtt gaacccttga atcaattcga agaaccaaac      420
accaaattctc gtcattgcgt acgcacgatg agaaaaccct aaccttggtg cccaagaag      480
ctgatataaa tctacgtcag atttccgtct agtcgtcctg atggatgaac ttgaggagga      540
t                                                                 541

<210> 28
<211> 318
<212> DNA
<213> Triticum aestivum

<400> 28
cctgatacat agttggcctg acgcgcatca attttagaa ctgatataaa aggggtgcag      60

```

9

cggctcgagt gccaggagca aggtccggaa gaagctcccc taaactgaga agagcacaca 120
 cacacacaca cacacgaact gacaagaaca cacacgaacg gacgctcact tgcgattgag 180
 aggcgaggca gacgaacaga taattagttt catgtcaaaa ttccagggat aattagtttc 240
 atgtcaaaat tccagggtatt cagcaacaat ggcacataag ttcagcattc attcatcagc 300
 gtcgccggga tccgggaa 318

<210> 29
 <211> 406
 <212> DNA
 <213> Triticum aestivum

<400> 29
 accattcttc ttcatagcca ctgacgaaga gtgcgacgat gaagatgatg atgatgatga 60
 tgatgatgat ggatgcattg ttcacaactt cgagttcacg caagcgagcc acctgacat 120
 attgcgcaca tgctagaggt cttgcttaga cttatcattg tcacattgcc atgcctcatg 180
 acaaaggtag atagttcatc tccgtgagga ggtcctgaga ggtcttcccg tgaggggtct 240
 tgaatccact tgggggatct tctctgtggg ccacggtctc taatggagaa agtatcaagt 300
 ggatgagcaa agctctatct ctaaaatgag atgaaccatt gctaacccta gaatggtgga 360
 ggtggatgag tataatatag ctagggttac gaagtggtaa gtgagg 406

<210> 30
 <211> 180
 <212> DNA
 <213> Triticum aestivum

<400> 30
 cgttgttctg gctagtgtct gacctccga agtggatcatg gcggctgtag cggagggtggc 60
 cacaccaatt gagaaggcag tctccggctg ttgttgctgt tgttggtgtt gttgttggtc 120
 atcctcatcc agcgaaataa agttctcatt cgaactcagc gccaaagctct ctttgggcag 180

<210> 31
 <211> 561
 <212> DNA
 <213> Triticum aestivum

<400> 31
 ccaatacacc attcatgttt tcttgtcaac taaagataat gatagtgtat attttttttag 60
 gaggaaaagt aaaagtaatg atagtctata ttataaacca acacactcgt gtggtttaca 120
 tgggttgctg tctatgcggc ccatgttgca cttgatagct ttttttagta tctgtactaa 180
 gtgcgtaaat tataatgata gtaataaaaa taacattcaa acattcgtgt gttataaata 240
 ttatacatac aaaagataac ttatttagca aaatgttcga gtaattttta aagtattgac 300
 gtcttatttt tgaatatata tatatataat agtatttctg attataaaaa aatgtttgta 360
 taatttatag tactacgaat atttccaaat tataactaat tattattatt attattatta 420
 ttattattga atatttgtat aattatatgc acattgtag aattcatgaa tttgttgttt 480
 aagtattttg tgtgatttaa aaatatccat tattatttag tatttaaact ttctataaat 540
 atagacttgt ggctatactt a 561

<210> 32
 <211> 393
 <212> DNA
 <213> Triticum aestivum

<400> 32
acgacctg gcacttttcc gacctcgctg gttcctagta gcagcgatag acaaattaaa 60
taaagggcta aactgattgg taacttaatt taccggggca aaaaagcaac tgggcctttg 120
tttctctcat gttcacagcc caacaatcaa caaacgacat tgctacatgc aatcagccca 180
ggccaccgaa acaaagaaac aaaggggagc tggcttcctt gggtcgctcc aactctctct 240
ctatctatct atctatctat ctatctatct atctctctct ctctctctct ctctctctg 300
tctctttgtt tctcacgcac gaccagcgca gggactgggc gacagagggc ggcatggcg 360
gatgaaccct agacggctcc ggcatcagcg gca 393

<210> 33
<211> 502
<212> DNA
<213> Triticum aestivum

<400> 33
tgaactaatc tcacagattc tgaaaagccg caggagccgt ccaccccaa taaatgagaa 60
cctaagaatg tcaaacacac tatggctagg ggcgcattcc ttgcaaggca ggctgcccga 120
acaatggctt tgggtgctga atctcgtgaa agaagatcag gaacacaaaa tcatgaaata 180
ataataataa taataataat aataataata ataataataa taataataat aataataata 240
ataataataa taataataat agacgaacca ctgataaaac caaatgtgac ctccacttg 300
ccgtcccat gacaagatga agcgtcggcc gataacctcg acgaccaagg agactatcac 360
actaggaagg agacaacacc acatgctcct cgatgtgtat cgcggcggtc ttgataatca 420
caccatgatg gagatagccg ttggacttgt atttctgtg acatggcctt aattgtcaac 480
atgccacaac cattgacata ta 502

<210> 34
<211> 479
<212> DNA
<213> Triticum aestivum

<400> 34
aattcgaact gaaactgaaa acacagacat cagagtttag aataataata ataataataa 60
taataataat aataataata ataataaaga acaaattgca aatgaaaaag attaaaaact 120
taaaattgat atttggcagg ggccgcgga acgcgtaccc cctctatcac aaattatgac 180
gtccactctc cctcaaaaagg gagatggtag gccagcacac ccacaaagt caatgtaggc 240
cactagccta ggccatggtt cttcttgatc agccatagac cccgtccagg taagtaagtt 300
tcaaggttgc ccaatgccct gctaatatag gatgcgctgc ctctgctacc tccttcgacg 360
ggcgatgtgg tactaaacga aacgaaagta tgcctcgcct ggtgctcgtc gcggctttcc 420
aggacgagga gttcgaacgg gccactgtgt atgggcctat tgcaccaagt accaatcgg 479

<210> 35
<211> 399
<212> DNA
<213> Triticum aestivum

<400> 35
agaaggcacc ggggcaacgc gcaacctcca aagtcaaata gatgggtaga cgacatagaa 60
gatgttagcc ggatagccat gccagtcggg caggccgaca aagggacccg atcatgaggt 120
ggccaacaac tgggaaggca tcagcatgac ccccccccc caccacaca gagagagaga 180
gagagagaga gagagagaga gagagagaga gcaccaatct agcagacca acggtccgga 240

acatctggtt agccacaaaa gggacccgac catgaggagg tcaacatccg gccaacatag 300
 caatcgaaact ccgccacata gccttcacca atgcatgaaa tgcacatgca acagcgaggc 360
 aaaagacgat agatgatagg gccctgaccg tgcacgtgg 399

<210> 36
 <211> 720
 <212> DNA
 <213> Triticum aestivum

<400> 36
 atatccttcg tatgttacat ttccaccaag ttttcaaata atgcttacac caagaataaa 60
 agttattttt ttgtacacc taaaatttat gttttcaaac aacttttgca ccaaaataag 120
 caaaaatatt gctgccacgt aacagaagcg gagaaagccc actgcaccat tgtggctcaa 180
 tagtggtaac tgttcgtata ataataataa caacttatta ttattattat tattacaaat 240
 tattattatt attattatta ttattattat tattattatt attaggcctt gcaatccatg 300
 gtgggacgtg gccaccacg ccaccctag ctccgccact gccacctaac actaaatgat 360
 tggtagctat ttccatattc ttatatatag taaaatatat gtatcttcat ttacacaaca 420
 actttttttc atatgaattg ttccacactt tagctatata ttgaagtttt catacattgt 480
 ttttcaaaa cgtaaattgc taacttataa tgatttcttt ttcttttcca tttacaaaat 540
 cgcggtgttt tctttgtcct ctatctaaga gtgtttttat acacgatcta tttcaaagt 600
 tgctttttat tggcatcaca tggaatccac atgaaaaagc aaacatataa ttttctcatt 660
 tttcttacat gtagcatcat atgtataaga tagttctatt atttatccac cttgcaagat 720

<210> 37
 <211> 339
 <212> DNA
 <213> Triticum aestivum

<400> 37
 cgtctttaca accaaccaat ctctacttc cacagettca cccatctgtc gtggctcgcc 60
 gacgcaatcg accccgtcag cggggagtgcc gccagcgccg caatcagccc ctogtgcgcc 120
 ggcaccacgt tcctctggtt atccgccacg ctccacagct ccaccgtctg cattatcaca 180
 tcgcatcaca tgtttgcctc tcttggtgta tacaccacct tattattatt attattatta 240
 ttattcagaa tttgcagcaa ccatgtcaat gcttgcttac ctggtaatca ccaacgatca 300
 aaactttcgg gtatctcgga tggaatatgc atgactggt 339

<210> 38
 <211> 331
 <212> DNA
 <213> Triticum aestivum

<400> 38
 ttggtatgat tggtttgcta tttaaaaaaa ggattgatct acgccgttat aattcgggtcc 60
 catcagatta acggctccta attactgatt aacgtaggaa tttatagaaa gtctgtaatt 120
 aatagaggta tagatagata tagatagata gatagataga tgtcaaattg atagaatttg 180
 catatataga atgttagatt aatgttactt tttcggggca tatagtattt gttctcgacg 240
 atatgtccgg ccgcctcct cgccgtcgac ccgttcgtga cgacgtcatg cttcagagga 300
 cccatgtcca ggactgggct ccgcgagct g 331

<210> 39
 <211> 419
 <212> DNA
 <213> *Triticum aestivum*

<400> 39
 accactttgt gtatatgttt taaattcaaa gtgaaagcgt aaacttacgg gaagaagcgt 60
 attgtgacgg tgaacccgac aaagtcaatc cgtgctttat tattattata tatatatata 120
 gggaaagata tatatatata tatatattat tattattatt attattacta ggacatatgc 180
 ccgtgcgttg caacgggaag gataaattgg tgtgcatctg caagcacaac accttgtgct 240
 tcggtacgaa gaaaacacta attgccccat ccttcaaaac gccacccac gcggtacgac 300
 atccggacgg tgccatcgcc ggcaggcagg cgcacgaatc gaggtttaga aaaattgtgg 360
 gggatcatgtt atactctctt tgcaaggat tattagactg gtggaccact tggctcag 419

<210> 40
 <211> 474
 <212> DNA
 <213> *Bacillus amyloliquefaciens*

<400> 40
 gtccaatctg cagccgtccg agacaggagg acatcgcca gctgaaaccg ggcagaatc 60
 cggccatttc tgaagagaaa aatggtaaac tgatagaata aaatcataag aaaggagccg 120
 cacatgaaaa aagcagtcac taacggggaa caaatcagaa gtatcagcga cctccaccag 180
 acattgaaaa aggagcttgc ccttccggaa tactacggtg aaaacctgga cgctttatgg 240
 gattgtctga ccggtgggtt ggagtaccg ctcgttttgg aatggaggca gtttgaacaa 300
 agcaagcagc tgactgaaaa tggcgccgag agtgtgcttc aggttttccg tgaagcgaaa 360
 gcggaaggct gcgacatcac catcatactt tottaatacg atcaatggga gatgaacaat 420
 atggaaacac aaacctcag ctatccgatt ggagaatatc agccgcctga atcg 474

<210> 41
 <211> 832
 <212> DNA
 <213> *Bacillus amyloliquefaciens*

<400> 41
 ctggaaaacg tcacattgct tccgcatatc gggtcagcaa cggctaaaat ccgcttgaat 60
 atgttcacac aagccgctca aaacatgatt gacgccgtat acggaagaac gccgaaaaac 120
 cttactaagg aatttcaata agaagaaaaa tcccggtttg ttccagccggg gtttattttt 180
 cgctagataa aaagtactat ttttaaatc tttctattcc tttctttcgt tgctgataca 240
 atgaaaagga atcagcttca catgatgaaa atgggaggta ttgctttgaa aaaacgatta 300
 tcgtggattt ccgtttgttt actggtgctt gtctccgagg cggggatgct gttttcaaca 360
 gctgcaaaa cggaaacatc ttctcacaag gcacacacag aagcacaggc tatcaacacg 420
 tttgacgggg ttgaggatta tcttcagaca tatcataagc tacctgataa ttacattaca 480
 aaatcagaag cacaagccct cggctgggtg gcatcaaaag ggaaccttgc agacgtcgct 540
 ccggggaaaa gcatcgccgg agacatcttc tcaaacaggg aaggcaaaact cccgggcaaa 600
 agcggacgaa catggcgatga agcggtatatt aactatacat caggcttcag aaattcagac 660
 cggattcttt actcaagcga ctgggtgatt taaaaacaa cggaccatta tcagaccttt 720
 aaaaaaatca gataacgaaa aaaacggctt ccctcgagg gccgtttttt tcagctttac 780
 ataaagtgtg taataaatit ttcttcaaac tctgatcggc caatttcact tt 832

<210> 42
 <211> 1023
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Gene conferring Hygromycin resistance

<220>
 <221> misc_feature
 <222> (1)..(3)
 <223> deoxyribonucleotide-triplet coding Lys

<220>
 <221> misc_feature
 <222> (4)..(6)
 <223> deoxyribonucleotide-triplet coding Lys

<220>
 <221> misc_feature
 <222> (1021)..(1023)
 <223> deoxyribonucleotide-triplet coding for STOP

```
<400> 42
nnnnnnccctg aactcaccgc gacgtctgtc gagaagtttc tgatcgaaaa gttcgacagc    60
gtctccgacc tgatgcagct ctccgagggc gaagaatctc gtgctttcag cttcgatgta    120
ggaggggctg gatatgtcct gcgggtaaat agctgcgccg atggttttcta caaagatcgt    180
tatgtttatc ggcactttgc atcggccgcg ctcccgattc cggaagtgtc tgacattggg    240
gaattcagcg agagcctgac ctattgcacg tcccgcctgt cacagggtgt cacgttgcaa    300
gacctgcctg aaaccgaact gcccgctgtt ctgcagccgg tcgcggaggc catggatgcg    360
atcgctgcgg ccgatcttag ccagacgagc gggttcggcc cattcggaacc gcaaggaatc    420
ggtcaataca ctacatggcg tgatttcata tgccgcgattg ctgatcccca tgtgtatcac    480
tggcaaatcg tgatggacga caccgtcagt gcgtccgtcg cgcaggctct cgatgagctg    540
atgctttggg ccgaggactg ccccgaaagc cggcacctcg tgcacgcgga tttcgggtcc    600
aacaatgtcc tgacggacaa tggccgcata acagcgggtca ttgactggag cgaggcgatg    660
ttcgggggatt cccaatacga ggtcgccaac atcttcttct ggaggccgtg gttggcttgt    720
atggagcagc agacgcgcta cttcgagcgg aggcacccgg agcttgagg atcgccgcgg    780
ctccgggctg atatgctccg cattgtgtct gaccaactct atcagagctt ggttgacggc    840
aatttcgatg atgcagcttg ggcgcagggt cgatgcgacg caatcgctcc atccggagcc    900
gggactgtcg ggcgtacaca aatcgccgcg agaagcgagg ccgtctggac cgatggctgt    960
gtagaagtac tagccgatag tggaaaccga cgcccagca ctctccgag ggcaaaggaa   1020
nnn                                                                    1023
```

<210> 43
 <211> 1525
 <212> DNA
 <213> Pseudomonas sp.

<220>
 <221> misc_feature
 <223> Gene Conferring glyphosate resistance

```
<400> 43
aattccgcat tgttgcgata cgccgtcatc cagagtgaag ggcacatact tctgtctatt    60
```

14

```

atcgggctcg gaaagcagct tcggcaaatac atgcaggtgg acgggcacaa ggccccgttc 120
caacgatgct tctatggcca ttctgagcgt ctgcggcgta atcgagagaa tagcgttcgg 180
gtccagtcga tctgtcgctt cttccggttt gacgtgatgc atcgtgaaga cgactccacg 240
tccaccggca gaagggaaga gcgcctcagc accggaaaag gcgatagcct ccagcccggc 300
ccggatcagc ggatatttca aactgccctt tatcgaccgc agcgacatgg cctgattctt 360
ttccccaaa ggctctacgg gaactgcctt gccccgttg cctatagcaa ttacgtcgcc 420
ccgctgaat tgcattcatc cattcacgcg gaaggccgca cgctgagcgt acgcttatac 480
atgctcttcg ttaacacatc aaagcgaagt cgaactcttc caccagtcgg ttccaccgtc 540
atgcgcatac tggtaaatct ctgccagctc ctcagcgctg aattccagct tcttcagggc 600
tcccagcggc taattttcaa gctgtgttac cgaacttgcc ccgatcagcg aagaggtgac 660
acgcgggtcg cgcagtgtcc aggcgatagc catttgagcc aatgtctgac cgcgacgttc 720
cgcaatggcg ttcagcgccc ggatgcgccc cagcagctct tcgttctgtg cactggcctt 780
cagggagccg cctgatctcg cccgcgcccc ttcggggatt ccgttcaaata agcgggaggt 840
cagcaatcct tgagccagag gcgaaaagac aatgcagccg gttccgatct cgccaagggt 900
atcgagcagg ccattttcga tccagcgatt gaacatgttg taattgggct gatgaatgaa 960
aagcggaacc cgctcttcct tcaggatcgc ggcagccttc tgcgtcagtt ccggcgaata 1020
gctggaaata cccacataaa gcgccttgcc ctgacggtgc agatgcgca gcgctcccat 1080
cgtctcttcc agcggcgctg tggcgctccac acggtgcgaa tagaagatgt cgacatagtc 1140
gagccccatc cgcttcagcg acctggtcga ggctggcgat caggatattg cgacctgccg 1200
ccaatgcctc cgtacggccc cggccacatg tccaaccgg cttttgaaga gatgatcatt 1260
tcacgcgat agggccgaaa atcactggcc atcatgcggc cgaagtttct ttcgctgag 1320
ccgggagcg gtccataatt gtttgcgagg tcgaagtggg tcacgccgag atgcaacgcc 1380
cgcgcagaat cgccggaccg ttttcataga catcgcgacc gccgaaattg tgccagagac 1440
caagcgaaat agctggcaga atcgatagcc gcttcgacca gtgcggcgat agatcatatc 1500
cgactgataa cggtcggagc tgcag 1525

```

```

<210> 44
<211> 2456
<212> DNA
<213> Mus musculus

```

```

<220>
<221> misc_feature
<223> GUS

```

```

<400> 44
agcctggcca gtatgtccct aaaatggagt gcgtgttggg tcgcgctggg ccagctgctg 60
tgcagctcg cgctggctct gaaggcgagg atgctgttcc cgaaggagag cccgtcgcgg 120
gagctcaagg cgctggacgg actgtggcac ttccgcgccg acctctcga caaccggctg 180
cagggtttcg agcagcaatg gtaccggcag ccgctacggg agtcgggccc agtcttgagc 240
atgcctgtcc cttctagctt caatgacatc acccaagaag cagcccttcg ggactttatt 300
ggctgggtgt ggtatgaacg ggaagcaatc ctgccacggc gatggacca agataccgac 360
atgagagtgg tgttgaggat caacagtgcc cattattatg cagttgtgtg ggtgaatggg 420
attcatgtgg tggaacatga gggaggtcac ctcccccttg aggtgacat tagcaagctg 480

```

gtccagagtg ggccctgac cacctgccgg atcacgattg ccatcaacaa cactgacc 540
 cctcatatcc ttccgccggg gaccatcgtc tacaagactg acacctccat gtatcccaag 600
 ggttactttg tccaggacac aagctttgac ttcttcaact atgcgggact gcacgatct 660
 gtggctctct ataccacccc taccacttac atcgatgata tcaactgtgat cactaatgtg 720
 gagcaagaca tcgggctggg gacctactgg atttctgtgc agggcagtga acatttccag 780
 ctagaagtgc aacttttgga tgaggggtgg aaagtcgtgg cccatgggac agggaaccag 840
 ggtcaacttc aggttcccag tgccaacctc tgggtggcctt acctgatgca tgagcatcca 900
 gcctacatgt actccttgga ggtgaagggtg acaacaactg agtctgtgac tgactactac 960
 acccttccca tcgggattcg aacagtggct gtcacaaaga gcaagttcct cataaacggg 1020
 aagcccttct atttccaagg ggtcaataag cacgaggatt cagatatccg agggaaaggc 1080
 ttcgactggc cgctgctggg aaaggatttc aacctgtccc gttggctcgg ggcaaattcc 1140
 tttcgtacca gccactatcc ctactcagag gaggtacttc agctctgtga ccgatacggg 1200
 atttgtgtca tcgatgagtg tcccggtgtg ggcattgtgc tacctcagag ttttggcaac 1260
 gagtcacttc ggcaccacct agaggtgatg gaggagctgg ttcgccggga caaaaatcac 1320
 cctgcggttg tgatgtgggc tgtggccaat gagccttccct ctgctctgaa acccgccgca 1380
 tattacttta agacgtgat caccacacc aaagccctgg acctcaccg tccgtgacc 1440
 tttgtgagca acgcaaaata tgatgcagac ctgggggccc cgtacgtgga tgttatctgt 1500
 gtaaacagct acttttcttg gtatcatgac tatgggcatt tggaggtgat tcagccacag 1560
 ctgaatagcc agtttgagaa ctggtataag acgcatcaga agccgattat ccagagcgag 1620
 tatggagcag acgcaatccc agggatccac gaggaccgc ctcgcatgtt cagtgaggag 1680
 taccagaagg ctgttctgga gaattacat tcagttctgg atcagaaacg taaagaatac 1740
 gtggtcggag agctcatctg gaatttcgcc gacttcatga cgaaccagtc accgctgaga 1800
 gtaatcgga acaagaaggg gatcttact cgccagagac agcccaaac ttcggccttt 1860
 attttgcgag agagatactg gaggattgcc aacgaaaccg gaggtcacgg ttcagggccg 1920
 agaaccagtg gtttcggaag cagaccgttc acgttctaaa gttacaacta cctcactgca 1980
 gactcaaagg tgtccccaga cacttctga cactagtgtt tctatttggg attttgtgaa 2040
 ctgatacaag gggactttag aagtgcacac aaagcttttc ttccgtgggg ataataactt 2100
 acagtgcctt aggtcagta atatgtcctg ctgagaggtg tccaagataa tttgttagtt 2160
 ttttgcctt ggctttgtga actcttgaaa gcctgctgtg tgaacattct cactaaatta 2220
 agagctactg gtgagaggct ggagtgaagg ctggctcaca atttaagagc actgactgtt 2280
 ctactggagg ttccagattc aattcctagc acccacctgg tggctaacta tgagctggtc 2340
 ctccatttcc caggtgatcc aatgccctt ttggcccctg cgggtaccac atgtatgtgg 2400
 tatgtggatc tacatgtggg gaaaaagtc atacatgcaa aataaaaatg aatcta 2456

<210> 45
 <211> 635
 <212> DNA
 <213> *Triticum aestivum*

<220>
 <221> misc_feature
 <223> WPG118

```

<400> 45
tcagtctgaa taagtacaga taatgcggtc ttatggcggg ggttgtttca ttgaagatga 60
ggactgggtca ttgtgggtcaa tgaatcgga aaattagtagc tttctctagc tttgcatgag 120
atctgtgaatt tgcaatcggt ttgttagttt tggccaaaca tatattgcag cttttggtca 180
gtggcgggagc tagctcccgg agacctgggg cagctgcccg ggctgctccg gccatccttc 240
actaaacaat acaaagaaac catgattagt attactaatt acttgggcaa atgctgggca 300
ctatcgccgt gtaaagattc tgccccggct ccttgagctg gctggctccg ccactgcttt 360
tggttctttc tctgcataaa gtacgccact gtttgctgaa caaattctca ttctaaagct 420
atggttactg atatgacaac gtagaactat catcgagata ttgatgtggt ctaaagcaac 480
cataaattgg actttcaata aatctgagta aattggaaga caattttaag tgggagtatg 540
cttaaattga tatctcagcc atactgtcca agcaacacca agatcatttg caacgagaaa 600
aagcacagaa caatgacctt atttatgccc cccct 635

```

```

<210> 46
<211> 235
<212> DNA
<213> Triticum aestivum

```

```

<220>
<221> misc_feature
<223> WPG90

```

```

<220>
<221> misc_feature
<222> (40)..(40)
<223> n is a, c, g, or t

```

```

<220>
<221> misc_feature
<222> (42)..(42)
<223> n is a, c, g, or t

```

```

<220>
<221> misc_feature
<222> (63)..(63)
<223> n is a, c, g, or t

```

```

<400> 46
tcgcaataat tagaaagaat ctaatacggt agtttgggcn cnacggatat gatggtcagt 60
tcntcttgat tgagtacca tcaatccgca ccaagttgag caaaatcaac aattttcagt 120
ccctatttgc tgcaattttt tagctagtaa acaaagcata tgaacagatc ttagcattcc 180
aaaaattaaa cttaacagat ctcagaaatc ttgttgagca agaaagaggc ctgtgt 235

```