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(54) Titre : BRASSICA CARINATA PRODUISANT DES SEMENCES A TENEUR REDUITE EN GLUCOSINOLATE
(54) Title: BRASSICA CARINATA PRODUCING SEED WITH REDUCED GLUCOSINOLATE CONTENT

(57) **Abrégé/Abstract:**

The invention is in the field of Brassica oilseed crops with novel traits, specifically to Brassica carinata plants carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to that of seed produced by a commercial or check Brassica carinata plant grown in the same location under the same environmental conditions. The present invention also relates to cell, seeds, methods, and uses of such Brassica carinata plants and their progeny.

ABSTRACT

The invention is in the field of *Brassica* oilseed crops with novel traits, specifically to *Brassica carinata* plants carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to that of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions. The present invention also relates to cell, seeds, methods, and uses of such *Brassica carinata* plants and their progeny.

***BRASSICA CARINATA* PRODUCING SEED
WITH REDUCED GLUCOSINOLATE CONTENT**

Cross-Reference to Related Applications

[0001] This application claims the benefit of and priority from United States Provisional Patent Application No. 62/677,241 filed May 29, 2018, United States Provisional Patent Application No. 62/677,243 filed May 29, 2018, United States Provisional Patent Application No. 62/677,244 filed May 29, 2018, and United States Provisional Patent Application No. 62/677,250 filed May 29, 2018, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of *Brassica* oilseeds, more specifically, to *Brassica carinata* plants carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to that of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

BACKGROUND

[0003] *Brassica carinata* is a member of the *Brassicaceae* (formerly *Cruciferae*) family, commonly known as the mustard family. In Canada, *Brassica carinata* is commonly known as carinata, but is also sometimes referred to as Ethiopian mustard, Abyssinian mustard, Ethiopian kale or Abyssinian kale. In Ethiopia it is named gomenzer (Getinet, *et al.*, 1996).

[0004] The genus *Brassica* is a member of the tribe Brassiceae in the mustard family (*Brassicaceae*; (Warwick, *et al.*, 2009). In addition to *B. carinata*, the *Brassica* genus includes several economically important oilseed crop species: *B. juncea* (L.) Czern. (brown mustard), *B. napus* L. (rape, Argentine canola), *B. nigra* (L.) W.D.J. Koch (black mustard), and *B. rapa* L. (field mustard, Polish canola). The genus *Brassica* also includes *B. oleracea* L. food crops, including cabbage, broccoli, cauliflower, brussels sprouts, kohlrabi and kale.

[0005] The six *Brassica* species are closely related genetically, as described in the Triangle of U (Nagaharu, 1935). *Brassica carinata* is an amphidiploid (BBCC, 2n=34) thought to be derived from interspecific hybridization of the diploid species *B. nigra* L. (BB, 2n=16) and *B. oleracea* L. (CC, 2n=18; (Prakash, *et al.*, 2011). The native range of *Brassica carinata* comprises the central highland region of Ethiopia. All the naturally occurring carinata in these regions is cultivated; there do not appear to be wild populations.

[0006] *Brassica carinata* is an herbaceous annual with a determinate growth habit (Zanetti, *et al.*, 2013). The plants were originally cultivated in their home range primarily as a source of leaves used as an edible and nutritious vegetable (Neugart, *et al.*, 2017). Carinata can be grown as a cover crop to reduce soil erosion and herbicide use, while promoting water conservation (Alcántara, *et al.*, 2011) or can be plowed into the soil for use as a green manure soil amendment and bio-fumigant (Lazzeri, *et al.*, 2009; Pane, *et al.*, 2013). Carinata also has utility in phytoremediation of heavy metal-contaminated topsoil (Mourato, *et al.*, 2015). As abundant producers of vegetative biomass, cropping of carinata's above-ground biomass has been suggested as a renewable source of feedstock for conversion to energy, particularly where cultivated in southern Europe (Gasol, *et al.*, 2007).

[0007] *Brassica carinata* can be grown in subtropical regions as winter cover crop in rotations with summer crops such as beans, cotton, peanuts, where the usual practice had been to follow with winter fallow and is made possible by the unique ability for established carinata to survive and recover after hard frosts (Seepaul, *et al.*, 2015). Benefits of carinata's use as a winter cover crop in this environment include the ability to conserve winter moisture and nutrients in the soil, mitigate leaching of nitrogen, phosphates and other residual nutrients into local waterways as well as providing a means to increase soil organic carbon (Newman, *et al.*, 2010 (revised)). *Brassica carinata*'s greater tolerance to early season frost, ability to better cope with higher heat and lower moisture during flowering and seed set as well as resistance to lodging, allows it to better withstand early and late season weather extremes (Seepaul, *et al.*, 2015), making it overall a more reliable oilseed cropping option for producers in semi-arid regions. *Brassica* crops have long been shown to be beneficial when grown in rotations with cereals such as wheat, an important food crop amenable to production in semiarid regions by virtue of its shorter growing season and tolerance to climate extremes. Rotations with oilseed as well as forage *Brassica* species have consistently demonstrated a beneficial effect on yield of the ensuing cereal crop,

due to their effects on improving soil structure and moisture conservation and to its ability to provide a break to the cycle of diseases that affect cereal performance (Angus, *et al.*, 2011). Its ability to break cereal disease cycles stems from *Brassica*'s lack of susceptibility to many cereal diseases, but may also derive from their ability to actively discourage persistence of soil pathogens via the biofumigant activity of root exudates and residues (Kirkegaard and Sarwar, 1998). In the southern hemisphere, the crop can be sown in late autumn or early winter into moist soil. In higher rainfall zones, it can be sown as late as early spring.

[0008] In terms of economic value, *Brassica carinata*'s greatest potential as a crop resides in its prolific yields of oil and protein rich seed. In southern Europe, carinata seed oil has been investigated for its potential as a feedstock for biofuel and as a bio-industrial feedstock with applications in production of lubricants, paints, cosmetics, plastics (Cardone, *et al.*, 2002; Cardone, *et al.*, 2003; Bouaid, *et al.*, 2005; Gasol, *et al.*, 2007; Gasol, *et al.*, 2009). In North America, where varieties have been adapted to grow in regions as diverse as the semi-arid southern Canadian prairies and adjacent US northern tier states as well as the Southeast US gulf states, carinata has been shown to be a suitable renewable feedstock crop for biofuel production (Gesch, *et al.*, 2015; Seepaul, *et al.*, 2015), and oil extracted from *B. carinata* seed has been used to produce green bio-diesel and bio-jet fuel (Drenth, *et al.*, 2015). In October 2012, experimental aviation flights by the National Research Council of Canada using the world's first 100% bio-jet fuel were successful ("ReadiJet 100% biofuels flight - one of 2012's 25 most important scientific events", Popular Science Magazine, 2012(12).

[0009] Carinata varieties have been developed that are optimized for production of oil feedstock for diverse bio-industrial uses such as manufacturing of bio-plastics (Impallomeni, *et al.*, 2011; Newson, *et al.*, 2014), lubricants (Zanetti, *et al.*, 2009) and specialty fatty acids such as 5, 13-docosadienoic acid, 5-eicosenoic acid (Jadhav, *et al.*, 2005), eicosapentaenoic acid (Cheng, *et al.*, 2010) and nervonic acid (Taylor, *et al.*, 2010). In some cases, modification of the seed oil profile has involved the use of transgenic technologies to introduce specific genes encoding enzymes of the fatty acid biosynthesis pathways or constructs to knock down expression of endogenous pathway genes (reviewed in Taylor, *et al.*, 2010).

[0010] As well as its high oil content, carinata seed has high protein and low fibre content (Xin and Yu, 2014), making the meal that is produced as a by-product of the oil extraction process a

potential source of high quality protein for use in animal feed applications. The native seed also has a high content of glucosinolate (GSL), a class of sulfur containing compounds which, when present at high levels in meal, can reduce feed palatability and adversely affect animal health.

[0011] *Brassica carinata* has been adapted to meet the demands of emerging markets for carinata seed and carinata products. To provide opportunity to expand its production base, there continues to be a great need in the art for new carinata plants with improved traits, including increased grain and oil yields per acre, increased seed oil content and optimized oil composition, reduced seed glucosinolate content, as well as improved agronomic traits such as increased tolerances to biotic and abiotic stresses, reduced time to maturity and improved harvestability.

SUMMARY OF INVENTION

[0012] In one aspect, the invention provides a cell, seed, or plant part of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions. In some embodiments, the total glucosinolate content of the seed of the *Brassica carinata* plant is reduced by at least 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 42%, 44%, 46%, 48%, or 50% relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0013] In other embodiments, the total glucosinolate content of the seed of the *Brassica carinata* plant is reduced by at least 8 $\mu\text{mol/g}$, 9 $\mu\text{mol/g}$, 10 $\mu\text{mol/g}$, 11 $\mu\text{mol/g}$, 12 $\mu\text{mol/g}$, 13 $\mu\text{mol/g}$, 14 $\mu\text{mol/g}$, 15 $\mu\text{mol/g}$, 16 $\mu\text{mol/g}$, 17 $\mu\text{mol/g}$, 18 $\mu\text{mol/g}$, 19 $\mu\text{mol/g}$, 20 $\mu\text{mol/g}$, 21 $\mu\text{mol/g}$, 22 $\mu\text{mol/g}$, 23 $\mu\text{mol/g}$, 24 $\mu\text{mol/g}$, 25 $\mu\text{mol/g}$, 26 $\mu\text{mol/g}$, 27 $\mu\text{mol/g}$, 28 $\mu\text{mol/g}$, 29 $\mu\text{mol/g}$, 30 $\mu\text{mol/g}$, 31 $\mu\text{mol/g}$, 32 $\mu\text{mol/g}$, 33 $\mu\text{mol/g}$, 34 $\mu\text{mol/g}$, 35 $\mu\text{mol/g}$, 36 $\mu\text{mol/g}$, 37 $\mu\text{mol/g}$, 38 $\mu\text{mol/g}$, 39 $\mu\text{mol/g}$, or 40 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0014] In one embodiment, the source from which the genetic determinant is acquired is formed by plants grown from seed of which a representative sample is deposited with the NCIMB under deposit number 43005 (*Brassica carinata* DH-146.047), 43006 (*Brassica carinata* DH-146.197), 43007 (*Brassica carinata* DH-146.214), or 43008 (*Brassica carinata* DH-146.8412), or from any cell, seed, plant part, or progeny thereof.

[0015] In another embodiment, the total glucosinolate content of the seed is no greater than 70 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed as determined by Near Infrared Spectroscopy (NIR) of mature seeds at less than 6% moisture, as recommended by the Western Canada Canola/Rapeseed Recommending Committee (WCC/RCC). The NIR is calibrated using a large array of samples with known GSL content determined previously by one or more methods including, but not limited to, gas chromatography of TMS-derivatives and HPLC of desulfoglucosinolates. In another embodiment, the total glucosinolate content of the seed is no greater than 65 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 60 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 55 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 50 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 45 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 40 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed.

[0016] In other aspects, the invention also provides a cell, seed, plant, or plant part of a *Brassica carinata* plant comprising a desired trait produced by crossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed of existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, with itself or with another *Brassica carinata* plant. In some embodiments, the invention provides

methods for producing a *Brassica carinata* plant by crossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, with itself or with other *Brassica carinata* plants.

[0017] In another aspect, the invention is directed to a *Brassica carinata* cell, seed, plant, or plant part produced by crossing *Brassica carinata* plants and harvesting the resulting *Brassica carinata* seed, wherein at least one *Brassica carinata* plant is a plant produced from a cell, seed, plant, or plant part of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0018] In another aspect, the invention is directed to a method of producing a *Brassica carinata* plant comprising (a) crossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, with a different *Brassica carinata* plant having a desired trait to produce F1 hybrid seed; and (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants having the desired trait and at least a portion of the genetic make up of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In one embodiment, the method further comprises the steps of (a) backcrossing the selected progeny plants with the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, or with the different *Brassica carinata* plant having a desired trait, to produce backcross progeny seed; (b) growing the resultant backcross progeny seed and selecting backcross progeny plants that have the desired trait and at least a

portion of the genetic make up of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions; and (c) repeating steps (a) and (b) to a maximum of 10 generations to produce a progeny *Brassica carinata* plant comprising the desired trait and producing seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check plant of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, steps (a) and (b) are repeated to a maximum of 10 generations to produce a progeny *Brassica carinata* plant comprising the desired trait and at least a portion of the genome of the *Brassica carinata* plant producing seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0019] In another embodiment, the method of producing a *Brassica carinata* plant derived from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions further comprises the steps of (a) self-pollinating the selected progeny plants to produce further progeny seed; (b) growing the further progeny seed and selecting further progeny plants that have the desired trait and at least a portion of the genetic make up of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions; and (c) repeating steps (a) and (b) to a maximum of 10 generations to produce a progeny *Brassica carinata* plant comprising the desired trait and producing seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check plant of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, steps (a) and (b) are repeated to a maximum of 10 generations to produce a progeny *Brassica carinata* plant comprising the desired trait and producing seed having a total

glucosinolate content that is reduced by at least 8 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check plant of *Brassica carinata* grown in the same location under the same environmental conditions.

[0020] In some embodiments, the total glucosinolate content of the seed produced by the progeny *Brassica carinata* plant will be further reduced relative to the total glucosinolate content of seed produced by the parent *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, the total glucosinolate content of the seed of the progeny *Brassica carinata* plant is further reduced by at least 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 42%, 44%, 46%, 48%, or 50%. In other embodiments, the total glucosinolate content of the seed produced by the progeny *Brassica carinata* plant is further reduced by at least 8 $\mu\text{mol/g}$, 9 $\mu\text{mol/g}$, 10 $\mu\text{mol/g}$, 11 $\mu\text{mol/g}$, 12 $\mu\text{mol/g}$, 13 $\mu\text{mol/g}$, 14 $\mu\text{mol/g}$, 15 $\mu\text{mol/g}$, 16 $\mu\text{mol/g}$, 17 $\mu\text{mol/g}$, 18 $\mu\text{mol/g}$, 19 $\mu\text{mol/g}$, 20 $\mu\text{mol/g}$, 21 $\mu\text{mol/g}$, 22 $\mu\text{mol/g}$, 23 $\mu\text{mol/g}$, 24 $\mu\text{mol/g}$, 25 $\mu\text{mol/g}$, 26 $\mu\text{mol/g}$, 27 $\mu\text{mol/g}$, 28 $\mu\text{mol/g}$, 29 $\mu\text{mol/g}$, 30 $\mu\text{mol/g}$, 31 $\mu\text{mol/g}$, 32 $\mu\text{mol/g}$, 33 $\mu\text{mol/g}$, 34 $\mu\text{mol/g}$, 35 $\mu\text{mol/g}$, 36 $\mu\text{mol/g}$, 37 $\mu\text{mol/g}$, 38 $\mu\text{mol/g}$, 39 $\mu\text{mol/g}$, or 40 $\mu\text{mol/g}$.

[0021] The invention is also directed to a cell, seed, plant, plant part of a progeny plant of the *Brassica carinata* plant derived from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, produced using the above-described methods.

[0022] In another aspect, the invention provides methods for producing doubled haploid (DH) F1 *Brassica carinata* plants produced by crossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, with itself or with other *Brassica carinata* plants, as well as to seeds and plants produced by such DH plants and

any progeny of these, especially progeny retaining the “essential morphological or physiological characteristics” of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, as described herein. In some embodiments, the “essential morphological or physiological characteristics” of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, are the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, as determined at the 5% significance level. In other embodiments, the total glucosinolate content of the seed produced such DH plants will be further reduced relative to the total glucosinolate content of seed produced by the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, the total glucosinolate content of the seed of such DH plants is further reduced by at least 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 42%, 44%, 46%, 48%, or 50%. In other embodiments, the total glucosinolate content of the seed of such DH plants is further reduced by at least 8 $\mu\text{mol/g}$, 9 $\mu\text{mol/g}$, 10 $\mu\text{mol/g}$, 11 $\mu\text{mol/g}$, 12 $\mu\text{mol/g}$, 13 $\mu\text{mol/g}$, 14 $\mu\text{mol/g}$, 15 $\mu\text{mol/g}$, 16 $\mu\text{mol/g}$, 17 $\mu\text{mol/g}$, 18 $\mu\text{mol/g}$, 19 $\mu\text{mol/g}$, 20 $\mu\text{mol/g}$, 21 $\mu\text{mol/g}$, 22 $\mu\text{mol/g}$, 23 $\mu\text{mol/g}$, 24 $\mu\text{mol/g}$, 25 $\mu\text{mol/g}$, 26 $\mu\text{mol/g}$, 27 $\mu\text{mol/g}$, 28 $\mu\text{mol/g}$, 29 $\mu\text{mol/g}$, 30 $\mu\text{mol/g}$, 31 $\mu\text{mol/g}$, 32 $\mu\text{mol/g}$, 33 $\mu\text{mol/g}$, 34 $\mu\text{mol/g}$, 35 $\mu\text{mol/g}$, 36 $\mu\text{mol/g}$, 37 $\mu\text{mol/g}$, 38 $\mu\text{mol/g}$, 39 $\mu\text{mol/g}$, or 40 $\mu\text{mol/g}$.

[0023] In another aspect, the invention provides methods for producing a *Brassica carinata* plant by outcrossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, with other *Brassicaceae* species, followed by backcrossing with a *Brassica carinata* plant carrying a genetic determinant that leads to

production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, as well as producing DH plants from the interspecific crosses. In some embodiments, the other *Brassicaceae* species may be any species of the family *Brassicaceae* including but not limited to *Brassica alba*, *Brassica hirta*, *Brassica juncea*, *Brassica napus*, *Brassica nigra*, *Brassica oleracea*, *Brassica rapa*, *Sinapis alba*, and *Camelina sativa*.

[0024] The invention also relates to the use of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, in a hybrid production scheme as described herein.

[0025] In another aspect, the invention provides for the use of a *Brassica carinata* carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, as a background for chemical and/ or radiation induced mutagenesis, for targeted gene editing, for modulation of traits via RNA interference or antisense RNA expression, or for introduction of traits via genetic transformation.

[0026] In another aspect, the invention provides a method for the production of a commercial crop of *Brassica carinata* plants carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, as well as any progeny plants derived therefrom. The invention also provides a method of producing a commercial plant product comprising growing the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, or any progeny plants derived therefrom, to produce a commercial crop and producing the commercial plant product from the commercial crop.

[0027] In another aspect, the invention provides for the use of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, to produce a commercial product, such as oil, meal, protein isolate, biofumigant, or crushed non-viable seed. In some embodiments, the commercial plant product comprises meal or protein isolate having a total glucosinolate content no greater than 30 micromoles per gram (dry weight) of seed.

[0028] The invention also provides for commercial products produced from a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, such as oil, meal, protein isolate, biofumigant, or crushed non-viable seed. In some embodiments, the commercial plant product comprises meal or protein isolate having a total glucosinolate content no greater than 30 micromoles per gram (dry weight) of seed.

[0029] The invention provides for, without limitation, the following numbered embodiments:

1. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
2. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 20% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
3. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 22% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

4. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 8 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
5. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 10 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
6. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 20 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
7. The cell of any one of embodiments 1 through 6, wherein the source from which the genetic determinant is acquired is formed by plants grown from seed of which a representative sample is deposited with the NCIMB under deposit number 43005, 43006, 43007, or 43008, or from any cell, seed, plant part, or progeny thereof.
8. A cell of a *Brassica carinata* plant comprising a desired trait produced by a method comprising the steps of:
 - (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7;
 - (c) backcrossing the selected progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with plants produced from cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce backcross progeny plants; and

- (d) growing seed from the resultant backcross progeny plants and selecting backcross progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7.
9. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
10. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 20% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
11. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 22% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
12. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
13. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 10 μmol per g of seed relative to the total glucosinolate content of seed produced by a

commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

14. The cell of embodiment 8, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 20 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
15. A cell of a *Brassica carinata* plant comprising a desired trait produced by a method comprising the steps of:
 - (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more F1 progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7;
 - (c) self-pollinating the F1 progeny plants that have the desired trait to produce further progeny plants;
 - (d) growing the resultant further progeny plants and selecting further progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7
16. The cell of embodiment 15, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
17. The cell of embodiment 15, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate

content that is reduced by at least 20% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

18. The cell of embodiment 15, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 22% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
19. The cell of embodiment 15 wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
20. The cell of embodiment 15, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 10 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
21. The cell of embodiment 15, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 20 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
22. A cell of a *Brassica carinata* plant comprising a desired trait, produced by a method comprising:

- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with a plant of another *Brassicaceae* species comprising the desired trait;
 - (b) producing F1 plants using embryo rescue techniques to recover viable F1 plants from the cross or growing F1 seeds;
 - (c) self-pollinating the F1 plants that have the desired trait and carinata character;
 - (d) producing F2 plants using embryo rescue techniques to recover viable F1 plants from the cross or growing F2 seeds;
 - (e) self-pollinating the F2 plants that have the desired trait and carinata character;
 - (f) producing progeny plants using embryo rescue techniques to recover viable F3 plants or growing F3 seeds;
 - (g) self-pollinating the progeny plants that have the desired trait and carinata character to produce further progeny plants; and
 - (h) selecting the progeny plants with the desired trait and carinata character.
23. The cell of embodiment 22, wherein steps (g) and (h) are repeated until the *Brassica carinata* plant has the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7.
24. A seed, plant, or plant part produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23.
25. A tissue culture of protoplasts or regenerable cells of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23.
26. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce seed, wherein the seed is produced by self-crossing or open pollination.

27. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce an F1 hybrid *Brassica carinata* seed, wherein the *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 is either a female parent or a male parent in a cross-fertilization.
28. A cell of an F1 hybrid plant grown from the F1 hybrid seed produced by the use of embodiment 7.
29. A cell of an F1 hybrid plant grown from the F1 hybrid seed produced by a method comprising crossing a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with a different *Brassica carinata* plant and harvesting the resultant F1 hybrid carinata seed.
30. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23 to produce a Double Haploid.
31. A cell, seed, plant or plant part of a Double Haploid produced by the use of embodiment 30.
32. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce a *Brassica carinata* plant comprising a desired trait, wherein the desired trait is introduced by crossing the *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with a plant of another *Brassica* species comprising the desired trait.
33. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23 to produce a *Brassica carinata* plant comprising a desired trait.
34. The use of embodiment 33, wherein the desired trait is conferred by a nucleic acid construct.
35. The use of embodiment 34, wherein the nucleic acid construct is introduced using polyethylene glycol (PEG) mediated nucleic acid uptake, electroporation, ballistic

infiltration using nucleic acid coated microprojectiles (gene gun), an *Agrobacterium* infiltration-based vector, or a plant virus-based vector.

36. The use of embodiment 34 or 35, wherein the nucleic acid construct comprises a transgene, an RNAi construct or an antisense RNA construct.
37. The use of embodiment 33, wherein the desired trait is introduced by exposing seedlings or microspores to a mutagenic agent.
38. The use of embodiment 37, wherein the mutagenic agent is ethyl methanesulfonate, N-ethyl-N-nitrosourea, or x-ray, gamma or ultraviolet radiation.
39. The use of any one of embodiments 32 to 38, wherein the desired trait is selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism.
40. The use of embodiment 39, wherein the desired trait is herbicide tolerance and the herbicide selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba.
41. Use of a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23 to produce a commercial crop.
42. Use of a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23 to produce a commercial plant product.
43. The use of embodiment 42, wherein the commercial plant product comprises oil, meal, protein isolate, or biofumigant.
44. The use of embodiment 43, wherein the commercial plant product comprises meal or protein isolate having a total glucosinolate content of no more than 30 micromoles per gram of meal (dry weight).
45. A cell of a *Brassica carinata* plant regenerated from the tissue culture of embodiment 25, wherein the plant has the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, as determined at the 5% significance level when grown in the same location under the same environmental

conditions as a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7.

46. A method of producing a *Brassica carinata* plant comprising a desired trait, the method comprising the steps of:
- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have the desired trait and at least a portion of the genetic make up of the plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7;
 - (c) backcrossing the selected progeny plants that have the desired trait with plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce backcross progeny seed; and
 - (d) growing the resultant backcross progeny seed and selecting backcross progeny plants that have the desired trait and at least a portion of the genetic make up of the plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7.
47. The method of embodiment 46, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
48. The method of embodiment 46, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

49. A method of producing a *Brassica carinata* plant comprising a desired trait, the method comprising introducing a DNA construct conferring the desired trait into a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23.
50. The method of embodiment 49, wherein the nucleic acid construct is introduced using polyethylene glycol (PEG) mediated nucleic acid uptake, electroporation, ballistic infiltration using nucleic acid coated microprojectiles (gene gun), an *Agrobacterium* infiltration-based vector, or a plant virus based vector.
51. The method of embodiment 49 or 50, wherein the nucleic acid construct comprises a transgene, an RNAi construct, or an antisense RNA construct.
52. The method of any one of embodiments 46 to 54, wherein the *Brassica carinata* plant comprises the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
53. The method of any one of embodiments 52 to 57, wherein the *Brassica carinata* plant comprises the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
54. A method of producing a *Brassica carinata* plant comprising a desired trait, the method comprising the steps of:
 - (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have the desired trait and at least a portion of the genetic make up of the plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7;

- (c) self-pollinating the progeny plants that have the desired trait and at least a portion of the genetic make up of the plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 to produce further progeny seed; and
 - (d) growing the further progeny seed and selecting further progeny plants that have the desired trait and at least a portion of the genetic make up of the plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7.
55. The method of embodiment 54, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
56. The method of embodiment 54, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
57. A method of producing a *Brassica carinata* plant comprising a desired trait, wherein the method comprises exposing seedlings or microspores plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 23 to a mutagenic agent and allowing the surviving fraction to develop into mature plants.
58. The method of embodiment 57, wherein the mutagenic agent is ethyl methanesulfonate, N-ethyl-N-nitrosourea, or x-ray, gamma or ultraviolet radiation.
59. A method of producing a *Brassica carinata* plant comprising a desired trait, the method comprising:
- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of embodiments 1 through 7 with a plant of another species of the family *Brassicaceae* comprising the desired trait;

- (b) producing F1 plants using embryo rescue techniques to recover viable F1 plants or growing F1 seeds;
 - (c) self-pollinating the F1 plants that have the desired trait and carinata character;
 - (d) producing F2 plants using embryo rescue techniques to recover viable F2 plants or growing F2 seeds;
 - (e) self-pollinating the F2 plants that have the desired trait and carinata character;
 - (f) producing F3 plants using embryo rescue techniques to recover viable F2 plants or growing F3 seeds;
 - (g) self-pollinating the progeny plants that have the desired trait and carinata character to produce further progeny plants; and
 - (h) selecting the progeny plants with the desired trait and carinata character to produce the *Brassica carinata* plant.
60. The method of embodiment 59, wherein steps (g) and (h) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
61. The method of embodiment 59, wherein steps (g) and (h) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
62. The method any one of embodiments 46 to 61, wherein the desired trait is selected from the group consisting of male sterility and/or fertility restoration, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism.

63. The method of embodiment 62, wherein the desired trait is herbicide tolerance and the herbicide is selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba.
64. A plant, plant part, cell, or seed of the *Brassica carinata* plant produced by the method of any one of embodiments 46 to 63.
65. A method for producing a *Brassica carinata* plant expressing a cytoplasmic male sterility (CMS) trait comprising the following steps:
- (a) using a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 as a pollen donor for crossing with an existing *Brassica* CMS plant, preferably a *Brassica napus* CMS plant or a *Brassica juncea* CMS plant, to produce viable F₁ CMS plants;
 - (b) backcrossing the F₁ CMS plants with the plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 as a pollen donor, to recover viable CMS BC₁ plants;
 - (c) repeatedly backcrossing CMS BC_x plants with the plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 using plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 as a pollen donor for up to six generations and recovering viable CMS BC₆ plants; and
 - (d) maintaining the *Brassica carinata* CMS line by crossing with plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 (used as B line) and harvesting of CMS seed.
66. A method for producing a *Brassica carinata* line expressing a fertility restorer function gene (Rf) comprising:
- (a) Using a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 in reciprocal crosses with an existing *Brassica* Rf plant, preferably a *Brassica carinata* Rf, whereby viable F₁ progeny plants are subsequently recovered then screened for presence of Rf gene marker and whereby Rf F₁ plants are selected;
 - (b) backcrossing the Rf F₁ plants with the plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23, whereby viable BC₁ plants are

subsequently recovered then screened for presence of Rf gene marker and whereby Rf BC₁ plants are selected.

- (c) repeated backcrossing of Rf BC_x plants with plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 for up to six generations, as described above, whereby, viable Rf BC₆ plants are ultimately recovered; and
- (d) maintenance of the *Brassica carinata* Rf line by self- crossing and harvesting of Rf seed from *Brassica carinata* Rf selfed parent.

67. A method for producing hybrid seed from crossing a female parent and a male parent, the method comprising the steps of:

- (a) crossing the plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 with another *Brassica carinata* plant;
- (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have a desired trait;
- (c) recovering the hybrid seed,

wherein the female parent is a *Brassica carinata* CMS line of plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23.

68. A method for producing hybrid seed from crossing a female parent and a male parent, the method comprising the steps of:

- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 with another *Brassica carinata* plant;
- (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have a desired trait;
- (c) recovering the hybrid seed,

wherein the male parent is a *Brassica carinata* CMS line of the plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23.

69. Hybrid seed produced from the method of any one of embodiments 65 to 68.

70. A method of producing a commercial crop, the method comprising growing a plurality of plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23.
71. A method of producing a commercial plant product, the method comprising growing a plurality of plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 to produce a commercial crop and producing the commercial plant product from the commercial crop.
72. The method of embodiment 71, wherein the commercial plant product comprises oil, meal, protein isolate, or biofumigant.
73. The method of embodiment 72, wherein the commercial plant product comprises meal or protein isolate having a total glucosinolate content of no more than 30 micromoles per gram (dry weight).
74. A commercial plant product produced by the method of any one of embodiments 71 to 73.
75. Oil, meal, protein isolate, or biofumigant produced by the method of any one of embodiments 71 to 73.
76. Crushed, non-viable seed of a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23.
77. A method for producing a group of cultivated plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 in a field, wherein harmful microorganisms are controlled by the application of a composition comprising one or more microbiocidal ingredient.
78. The method of embodiment 77, wherein the composition comprising one or more microbiocidal ingredient is applied as a seed treatment or seed coating.
79. A method for producing a group of cultivated plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23 in a field, wherein insect pests are controlled by the application of a composition comprising one or more insecticidal ingredient.
80. A method for cleaning seed produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23, to remove foreign material from the surface of the seed.

81. A method for cleaning seed produced from the cell of a *Brassica carinata* plant of any one of claims 1 to 23, to remove any debris or low quality, infested, or infected seeds, or seeds of different species.

DEFINITIONS

[0030] In the following description and tables, many terms are used. To aid in a clear and consistent understanding of the specification, the following definitions and evaluation criteria are provided.

[0031] **Abiotic stress** is defined as the negative impact of non-living factors on the living organisms in a specific environment. Examples of abiotic stress include, but are not limited to, drought, water -logging or flooding, extreme temperatures, extreme salinity, and mineral toxicity.

[0032] **Agronomic practice** refers to any of a set of cultivation practices or techniques that attempt to maximize the health and productivity of a crop. The agronomic practices used are an important factor in interpreting results from field studies of various kinds.

[0033] **Allele** refers to one or more alternative forms of a gene locus that relate to one trait. Diploid organisms, i.e., organisms with two sets of chromosomes, have one copy of each gene (and therefore, one allele) on each chromosome. If both the alleles are the same, they are homozygous. If the alleles are different, they are heterozygous.

[0034] **Alternaria resistance** is typically rated on a scale from 0-5; 0= no symptoms were observed, 1= indicates infection of pods only, 2= disease prevalence on 25% of upper plant, 3= 50% prevalence, 4=75% prevalence, and 5= disease symptoms are seen throughout entire plant and considered severe.

[0035] **Average** refers to the arithmetic mean. “Substantially equivalent” or “statistically equivalent” refers to a characteristic that, when compared, does not show a statistically significant difference from the mean. In contrast, “statistically different” or “statistical significance” refers to a characteristic that, when compared, shows statistically significant differences from means of the same characteristic of another group or groups. Most often, statistical significance of differences is measured at levels of $P < 0.05$ using standard tests to compare Least Square Means, such as Tukey’s HSD or Student’s t-test.

[0036] **Backcrossing** means a traditional breeding technique used to introduce a trait to a plant line or variant from a donor plant to a recurrent plant. An initial cross is made between the donor and recurrent parent plants to produce progeny plants. Progeny plants having the desired trait are then crossed to the recurrent parent. This process of backcrossing is repeated for several breeding cycles until the progeny plants are indistinguishable from the recurrent parent, except for the trait from the donor parent.

[0037] **Breeding line (or Plant line)** refers to a unique, reproducible carinata type, and is distinguishable from other carinata types based on its genotype and phenotype. Most often, a breeding line refers to a type that is mostly or completely homozygous, such as a DH line or a highly inbred line (five or more generations inbred).

[0038] **Carinata** refers to seeds or plants of the species *Brassica carinata* containing both the B genome from *Brassica nigra* and the C genome from *Brassica oleracea* (Nagahuru, 1935). The terms “*Brassica carinata* DH-146.047” and “DH-146.047” are used interchangeably herein and refer to a plant of *Brassica carinata* DH-146.047, representative seed of which having been deposited under NCIMB Accession number 43005. The terms “*Brassica carinata* DH-146.194”, and “DH-146.842” are used interchangeably herein and refer to a plant of *Brassica carinata* DH-146.842, representative seed of which having been deposited under NCIMB Accession number 43006. The terms “*Brassica carinata* DH-146.214”, and “DH-146.214” are used interchangeably herein and refer to a plant of *Brassica carinata* DH-146.214, representative seed of which having been deposited under NCIMB Accession number 43007. The terms “*Brassica carinata* DH-146.842” and “DH-146.842” are used interchangeably herein and refer to a plant of *Brassica carinata* DH-146.842, representative seed of which having been deposited under NCIMB Accession number 43008.

[0039] **Check variety (or Check line)** are carinata genotypes considered to be the standard for overall performance, or for a specified trait, in a given growing region. Often, the current commercial varieties are used as check lines for the regions in which they are grown, and this is the standard against which new potential varieties are tested. Examples of useful commercial check varieties include, but are not limited to, *Brassica carinata* AAC-A120 and AGR044-312D-HP11 (WO2017/181276A1; henceforth referred to as “AGR044-HP11” or “HP11”)

[0040] **Days to Flowering (initiation of flowering)** refers to the number of days from planting until 50% of the plants in a planted area have at least one open flower.

[0041] **Depth of canopy**, measured in cm, is the distance from the first incidence of pods on a plant (average within a plot) to the top of the pod canopy.

[0042] **Diploid** refers to cell or a plant with two sets of chromosomes. One set comes from each parent.

[0043] **Double Haploid, Doubled Haploid, Doubled Haploidy (DH)** refers to a haploid cell or plant that has undergone a doubling of its chromosomes to produce a functional diploid.

[0044] **Duration of flowering** is the number of days between the initiation of flowering and end of flowering.

[0045] **Early (season) vigour** is a rating, usually on a scale of 1 to 7, that reflects how a variety develops in its early stages (about 4 weeks after seed) in terms of putting on leaf area and competing with weeds. Typically, experimental varieties or lines are compared with a check variety or line, which is rated as a "4". A new variety with slightly more leaf cover and advanced development would be rated a "5", or more significantly a "6", and so forth. 1 = significantly less than check variety; 7 = significantly greater uniformity than check variety.

[0046] **End of flowering** is the number of days from planting to when approximately 90% of flowers have lost their petals.

[0047] **Erucic Acid content** is the weight percentage (wt%) of fatty acids in the form of C22:1 among all major fatty acid types found in carinata. Most often, this is estimated by Near Infrared Spectroscopy (NIR) of mature seeds at less than 6% moisture. The NIR is calibrated using a large array of samples whose fatty acid profile is determined by American Oil Chemists Society (AOCS) Official Method Ce1-66 Fatty Acid Composition by Gas Chromatography. This is one of the official methods recommended by the Western Canada Canola/Rapeseed Recommending Committee (WCC/RCC).

[0048] **Extent of branching** refers to the number of pod-bearing secondary branches (racemes) off the main stem. This is often estimated using an average of at least eight plants per breeding line.

[0049] **Embryo Rescue techniques** refers to *in vitro* techniques whose purpose is to promote the development of an immature or weak embryo into a viable plant. This methodology is commonly used to rescue viable embryos from crosses between different but closely related species, *i.e.*, interspecific crosses.

[0050] **Fatty acid content** means the typical percentages by weight of fatty acids present in the endogenously formed oil of the mature whole dried seeds, as determined by Near Infrared Spectroscopy at less than 6% seed moisture. The NIR is calibrated using a large array of samples whose fatty acid profile is determined by American Oil Chemists Society (AOCS) Official Method Ce1-66 Fatty Acid Composition by Gas Chromatography. This is one of the official methods recommended by the Western Canada Canola/Rapeseed Recommending Committee (WCC/RCC). Aside from erucic acid (C22:1), the most significantly occurring fatty acids in carinata are oleic acid (c18:1), linoleic acid (c18:2), linolenic acid (c18:3), and eicosenoic acid (C20:1).

[0051] **Flower Petal Colouration** means the colouration of open exposed petals on the first day that flowering is observed. In carinata, varieties are most often categorized as either W= White or Y= Yellow. Occasionally, for varieties with a very light yellow colour, a third category will be employed: PY= Pale Yellow.

[0052] **Frost tolerance** means the ability of young plants to withstand frosts in areas where carinata is grown during the winter season. These frosts are most likely to occur from the five-leaf to early bolting plant development stages, depending on time of planting. This is typically measured 3 – 5 days following a hard frost event and rated using a scale of visual percentage of injury (leaf bleaching and death); where “0” is no injury and “10” is 100% plant damage, and each successive number indicates an additional 10% injury to leaf and stem tissue.

[0053] **Gene silencing** means the interruption or suppression of the expression of a gene at the level of transcription or translation.

[0054] **Genotype** refers to the genetics or DNA sequence of individual carinata lines, as opposed to their actual appearance, which is called the phenotype.

[0055] **Glucosinolate (GSL) content** means the total glucosinolate content of mature seed at less than 6% moisture, given in units of $\mu\text{mol/g}$, as analyzed using Near Infrared Spectroscopy.

In carinata, the majority of glucosinolate content is in the form of sinigrin (chemical name: allylglucosinolate or 2-propenylglucosinolate). Most often, the total glucosinolate content of seeds is estimated by Near Infrared Spectroscopy (NIR) of mature seeds at less than 6% moisture. The NIR is calibrated using a large array of samples with known GSL content determined previously by one or more methods known in the art including, but not limited to, gas chromatography of TMS-derivatives, and HPLC of desulfoglucosinolates.

[0056] **Grain** means the seed produced by carinata crops that are intended for processing for oil or feed uses. This is in contrast with parent seed or planting seed, which is intended for growth of another generation of plants.

[0057] **Growing Degree Days** refers to the accumulation of heat units above a base temperature over time and is often strongly correlated with rate of plant development. The daily GDD units are calculated by subtracting the average temperature (°C) (average of daily maximum and minimum temperature) from the baseline of 5°C. These units accumulate beginning the day after planting.

[0058] **Growing Environment** refers to a particular area (typically grouped by geography) or controlled environment where plants are grown and evaluated. Expression of various characteristics or traits, such as plant height or days to maturity, can be greatly influenced by the environment in which it is grown. This is also commonly known as **Genotype x Environment interaction, or G x E**.

[0059] **Haploid** refers to a plant, or a cell of a plant, that has only one set of chromosomes. Haploid plants can be produced artificially, and their single set of chromosomes can be doubled to produce a doubled haploid.

[0060] **Height of first pod**, measured in cm, refers to the distance from ground level to the first incidence of pods on a plant (average within a plot).

[0061] **Herbicide Resistance** means the resistance to various herbicides when that herbicide is applied at standard recommended application rates and timing; and is expressed on a scale of 0 to 10; where “0” is no symptoms, and a rating of “10” means entire plant is brown and curled. Each successive number indicates approximately 10% more damage or effect of herbicide on the plant: a rating of “2” indicates several leaves with slight yellowing and curl, “4” is several

yellowing or dying leaves and more noticeable curl, “6” is a greater number and severity of yellowing, curling, and dead leaves, and “8” indicates that most leaves are completely yellow or dead.

[0062] **Hybrid or F1 hybrid** refers to the seeds harvested from crossing two inbred (nearly homozygous) parental lines. This is most often accomplished in carinata through the development of inbred parents using the Ogura pollination control system, where one parent is functionally male sterile (CMS or A-line) and the other restores fertility to the F1 hybrid (Rf or R-line).

[0063] **Leaf colour** means the colour of the leaf blade of lower leaves at the late bolting to early flowering stages (on a plot basis). A categorical scale is most often used, where DG = Dark green; G = Green; LG = Light green; BG = Bluish green.

[0064] **Leaf glaucosity** refers to the presence or absence of a fine whitish powdery coating on the surface of the leaves, and the degree thereof, when present. Numerical scale used to rate this trait: 3= weak, 5=medium, 7=strong.

[0065] **Leaf Length** is the total length of the leaf from attachment of the petiole to the stem, to the tip of the blade, measured in cm, of lower leaves at the late bolting to early flowering stages. It is estimated using an average of measurements from at least 30 plants.

[0066] **Leaf number of Lobes** means the frequency of leaf lobes, when present, of lower leaves at the late bolting to early flowering stages. 3=few, 5=medium, 7=many.

[0067] **Leaf Width** is the width, in cm, of the widest part of the leaf blade of lower leaves at the late bolting to early flowering stages. It is estimated using an average of measurements from at least 30 plants.

[0068] **Locus** refers to a specific location in a chromosome.

[0069] **Lodging**, is the displacement of stems from their vertical and proper placement, observed as extent to which plants remain upright; upright plants are easier to harvest with harvesting equipment. In some trials, lodging is measured on a scale of 1-5, where 1 = significantly less lodging than check; 5 = significantly greater lodging than check. In other trials, lodging is measured on a scale of 1-7, where 1 = plot flat on the ground; 2 = most of plot on ground; 3 =

75% lodged; 4 = 50% lodged; 5 = 25% lodged; 6 = only slight lodging / stem bending noticed; 7 = no lodging.

[0070] **Maturity** may be determined based on Seed Maturity, Physiological Maturity or both.

[0071] **Near Infrared Spectroscopy (NIR)** refers to a non-destructive, spectrometric method commonly used to assess seed quality parameters in grain producing crops. Estimates of total seed oil content and fatty acid profile, as well as total glucosinolate and protein content on a whole seed basis, are examples of parameters for which NIR is commonly employed.

[0072] **Number of seed-bearing pods** is determined by counting the number of pods containing at least one viable seed on the main raceme, as measured in at least eight plants of the same line at full pod set stage.

[0073] **Oil content** means the typical percentage by weight (wt%) of oil present in the mature whole dried seeds, at less than 6% moisture, as determined near infrared (NIR) spectroscopy (AOCS Procedure Am 1-92 Determination of Oil, Moisture and Volatile Matter, and Protein by Near-Infrared Reflectance).

[0074] **Open-pollinated (OP) variety** refers to varieties that are maintained and propagated primarily by means of self-pollination, and for which the next generation will be largely equivalent to the previous.

[0075] **Pedicle Length** is the typical length of the silique stem observed when mature, measured in mm, for 30 pods taken per plot, from pods occurring on mid-third of the main raceme.

[0076] **Petiole Length** is the length of the petiole, measured in cm, from attachment of the petiole to the stem, to the start of the leaf blade, as observed in lower leaves at the late bolting to early flowering stages.

[0077] **Phenotype** refers to the outward appearance or manifestation of given traits of varieties, individual plants, or plant parts (such as leaves or seeds).

[0078] **Physiological maturity** means the number of days from planting to the stage when pods with seed change colour, from green to a dry tan look and when about 70-80% of stems and pods dry-down, corresponding to stages 90 and beyond of the BBCH scale (Meier, *et al.*, 2009).

[0079] **Plant** includes the whole plant, or any plant parts such as plant organs, plant cells, plant protoplasts, plant cell cultures, or plant tissue cultures from which whole plants can be regenerated, plant callus, plant cell clumps, plant transplants, seedlings, plant cells that are intact in plants, plant clones or micropropagations.

[0080] **Plant height** means the overall plant height from ground level to the average of the top of the plant canopy, in cm, taken between mid-flowering and full pod-set stages of plant development.

[0081] **Plant length** is the length of the plant, in cm, measured at maturity. For this trait, it is typically an average 30 or more plants measured individually from the same variety.

[0082] **Plant part** includes, but is not limited to, harvested tissues, fruits, organs, plant cuttings, vegetative propagations, embryos, pollen, ovules, flowers, leaves, fruits, fruit flesh, seeds, clonally propagated plants, roots, stems, stalks, root tips, grafts, parts of any of these and the like, or derivatives thereof, preferably having the same genetic makeup (or very similar genetic make up) as the plant from which it is obtained. Plant part also includes any developmental stage, such as seedlings, cutting prior or after rooting, mature and/or immature plants, or mature and/or immature leaves.

[0083] **Ploidy** refers to the number of sets of chromosomes exhibited by the line, for example, diploid (two sets) or tetraploid (four sets).

[0084] **Plot** refers to a group of carinata plants, grown in one or multiple rows of varying lengths, which is often used as the basic unit of measurement for a number of traits. These are often, but not always, associated with replicated yield trials.

[0085] **Pod shatter loss** is the amount of seed lost from shattered pods at harvest; seeds are collected in three 7" × 13" pans placed 3' from either end or at center of each plot and converted to kg/ha.

[0086] **Pod shatter resistance, or shatter resistance**, means the resistance to silique shattering observed at seed maturity. 1= not tested, 3= poor, 5= fair, 7= good, 9- does not shatter.

[0087] **Pod (silique) Beak length**, measured in mm, for 30 pods taken per plot, from pods occurring on mid-third of the main raceme.

[0088] **Pod (silique) length** is the length of the pod not including the pedicel or beak, measured in mm, for 30 pods per plot taken from the mid-third of the main raceme.

[0089] **Pod (silique) width** is the typical pod width when mature, for 30 pods per plot taken from the mid-third of the main raceme. Rating scale is typically categorical: 3= narrow; 5= medium, 7= broad.

[0090] **Primary raceme length**, measured in cm, means the measurement of the main raceme from the last (youngest) branch to the top of the inflorescence. This measurement often taken from end flowering to full pod set stage of plant development.

[0091] **Progeny** refers to plants derived from a plant of *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions. Progeny may be derived by regeneration of cell culture or tissue culture, self-pollination, or crossing at least one plant of *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions, with another plant of *Brassica carinata* or other *Brassicaceae* species, or by producing seeds of a plant of *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced glucosinolate content relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.

[0092] **Protein content** means the typical percentage by weight (wt%) of protein in the oil-free meal of the mature whole dried seeds, at less than 6% moisture, analyzed using near infrared (NIR) spectroscopy (AOCS Procedure Am 1-92 Determination of Oil, Moisture and Volatile Matter, and Protein by Near-Infrared Reflectance).

[0093] **Randomized Complete Block Design (RCBD)** is the most common experimental design used in standard replicated yield testing, typically consisting of four replications of various breeding lines or varieties, having the complete set of entries arranged in different randomizations for each replication.

[0094] **Recovery from frost damage**, is assessed by rating the same plots at a couple additional time points beyond the initial rating, often at two and three weeks following a hard frost event.

[0095] **Regeneration** means the development of a plant from cell culture or tissue culture or vegetative propagation.

[0096] **Replication** refers to a series of ratings or observations, taken from different plots or samples of the same variety, breeding line, or trait thereof, from plants grown at the same location or within a given set of trials. Replicated measurements are critical for greater accuracy in statistical analysis.

[0097] **Resistance** means the ability of a plant to withstand exposure to an insect, disease, herbicide or other potentially stress-inducing condition. A resistant plant variety or hybrid will have a level of resistance higher than a comparable check variety or hybrid.

[0098] **Saturated Fatty Acids** refer to Fatty Acids in which carbon chains are linked by single bonds; they lack unsaturated links between carbon atoms. The Saturated Fatty Acid content of oil in the seed is

[0099] **Sclerotinia resistance** is typically rated on a scale from 0-5; 0= no symptoms were observed, 1= infection of pods only, 2= disease prevalence on 25% of upper plant, 3= 50% prevalence, 4= 75% prevalence, and 5= disease symptoms are seen throughout entire plant and considered severe.

[0100] **Seed colour (NIR)**, means the seed coat colour of typical mature seeds based on NIR analysis. For this objective measurement, the colour of an aggregate sample of seed (1-5 g of seed) is determined by using the FOS XBR, or equivalent, near infrared spectrophotometer as a reflectance spectrophotometer over the visible range (400 nm to 700 nm) to provide an objective description of the carinata seed color, essentially as described (Black and Panozzo, 2004) except that seed color is described in terms of the Hunter L*A*B* color space rather than CIEL*A*B* color space.

[0101] **Seed colour (visual)**, is a subjective categorization of the predominant seed coat colour at seed maturity for a given variety or breeding line. These are generally grouped into the following categories: Y= Yellow or bright yellow; DY = Dark yellow; LB = Light brown; B = Brown; DB = Dark brown; O = Orange.

[0102] **Seed Maturity** refers to number of days from planting to the stage when 70-80% seeds on main raceme had seeds with complete colour change and hard when pressed between fingers.

[0103] **Seeds per pod**, means the average number of seeds per pod, in at least 30 pods taken from the mid-third of the main stalk.

[0104] **Seed weight (thousand seed weight, or TKW)**, means the weight, in grams, of 1,000 typical seeds determined at maturity, as measured at a seed moisture content of approximately 5-6%.

[0105] **Self-pollination or “selfing”** means the self-pollination of a plant by transfer of pollen from an anther to a stigma of the same plant. Carinata is a **self-compatible** species, meaning there are no genetic or physiological impediments to successful self-pollination.

[0106] **Stand** is the number of plants counted in 4 rows and converted to plants/m². Recorded 2-4 days before harvest.

[0107] **Stem colour** refers to the predominant stem colour at bolting stage, depending on levels of anthocyanin manifest in the stem. This is generally categorical, characterized as G = Green; LP = Light Purple; and P = Purple or dark purple.

[0108] **Tissue culture** refers to a composition comprising isolated cells of the same or a different type or a collection of such cells organized into part of a plant. This technique is an integral part of breeding techniques such as doubled haploidy, whereby new diploid plants of a unique, homozygous genetic composition are generated in lab conditions to the point where normal plant growth can occur in the field or in a greenhouse.

[0109] **Tolerance** is commonly used in the context of plants affected by abiotic stress, diseases, or pests and is used to describe an improved level of field resistance.

[0110] **Traditional plant breeding techniques** include, but are not limited to, crossing, selfing, selection, double haploid production, embryo rescue, marker assisted selection, mutation breeding, backcross breeding, single seed descent, and any other method known to the breeder other than genetic modification and transformation/transgenic methods, by which a genetically heritable trait can be transferred from one carinata line or variety to another, or traits of interest fixed in one genetic background.

[0111] **Trait introgression** refers to plants within a variety have been modified in a manner that retains the overall genetics of the variety and further comprises one or more loci with a specific desired trait, such as male sterility, disease, or herbicide resistance. Backcrossing followed by inbreeding or DH line generation is a common methodology to achieve this objective. Using this technique, one or more value added traits may be introduced into a single carinata variety.

[0112] **Transgene** means a genetic locus comprising a DNA sequence which has been introduced into the genome of a *Brassica carinata* plant by transformation. A plant comprising a transgene stably integrated into its genome is referred to as a transgenic plant.

DETAILED DESCRIPTION

[0113] In one aspect, the present invention relates to a cell, seed, plant or plant part of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions is provided. The total glucosinolate content of the seed of the *Brassica carinata* plant may be reduced by at least 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 42%, 44%, 46%, 48%, or 50% relative to total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* in the same location under the same environmental conditions. In one embodiment, the total glucosinolate content of the seed is reduced by at least 14% relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, as determined by Near Infrared Spectroscopy (NIR) analysis of harvested sees. In another embodiment, the total glucosinolate content of the seed is reduced by at least 20% relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* in the same location under the same environmental conditions. In another embodiment, the total glucosinolate content of the seed is reduced by at least 25% relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* in the same location under the same environmental conditions. In another embodiment, the total glucosinolate content of the seed is reduced by at least 30% relative to the total glucosinolate content of seed produced by existing commercial or check

plants of *Brassica carinata* grown in the same location under the same environmental conditions. In another embodiment, the total glucosinolate content of the seed is reduced by at least 35% relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In another embodiment, the total glucosinolate content of the seed is reduced by at least 40% lower relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* in the same location under the same environmental conditions.

[0114] In other embodiments, the total glucosinolate content of the seed of the *Brassica carinata* plant may be reduced by least 8 $\mu\text{mol/g}$, 9 $\mu\text{mol/g}$, 10 $\mu\text{mol/g}$, 11 $\mu\text{mol/g}$, 12 $\mu\text{mol/g}$, 13 $\mu\text{mol/g}$, 14 $\mu\text{mol/g}$, 15 $\mu\text{mol/g}$, 16 $\mu\text{mol/g}$, 17 $\mu\text{mol/g}$, 18 $\mu\text{mol/g}$, 19 $\mu\text{mol/g}$, 20 $\mu\text{mol/g}$, 21 $\mu\text{mol/g}$, 22 $\mu\text{mol/g}$, 23 $\mu\text{mol/g}$, 24 $\mu\text{mol/g}$, 25 $\mu\text{mol/g}$, 26 $\mu\text{mol/g}$, 27 $\mu\text{mol/g}$, 28 $\mu\text{mol/g}$, 29 $\mu\text{mol/g}$, 30 $\mu\text{mol/g}$, 31 $\mu\text{mol/g}$, 32 $\mu\text{mol/g}$, 33 $\mu\text{mol/g}$, 34 $\mu\text{mol/g}$, 35 $\mu\text{mol/g}$, 36 $\mu\text{mol/g}$, 37 $\mu\text{mol/g}$, 38 $\mu\text{mol/g}$, 39 $\mu\text{mol/g}$, or 40 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0115] The present invention also relates to any plants produced or derived from cells, seeds, plants, and plant parts of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, that exhibit “carinata character”.

[0116] For the purpose of the present invention, plants exhibiting “carinata character” have an erect, upright bearing, are highly branching, with well-developed and aggressive tap root systems (Barro and Martín, 1999). Leaves are generally wide elliptic in shape with weak-medium dentation, medium glaucosity, and very sparse pubescence. Seeds are globose, 1-1.5 mm in diameter and finely reticulated (Mnzava and Schippers, 2004) and vary from yellow to yellow-brown to brown in colour (Getinet 1987; Rahman and Tahir, 2010).

[0117] Plants exhibiting “carinata character” may be produced from a cell, seed, plant or plant part of *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the

same environmental conditions, using one or more (conventional) plant breeding technologies, cell culture or tissue culture technologies, and/or transgenic technologies.

[0118] In another aspect of the invention, the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions may be *Brassica carinata* DH-146.047 (representative seed of which having been deposited under NCIMB accession number 43005), *Brassica carinata* DH-146.194, (representative seed of which having been deposited under NCIMB accession number 43006), *Brassica carinata* DH-146.214 (representative seed of which having been deposited under NCIMB accession number 43007), or *Brassica carinata* DH-146.842 (representative seed of which having been deposited under NCIMB accession number 43008).

[0119] In another embodiment, the total glucosinolate content of the seed is no greater than 70 $\mu\text{mol/g}$ as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed as determined by Near Infrared Spectroscopy (NIR) of mature seeds at less than 6% moisture, as recommended by the Western Canada Canola/Rapeseed Recommending Committee (WCC/RCC). The NIR is calibrated using a large array of samples with known GSL content determined previously by one or more methods including, but not limited to, gas chromatography of TMS-derivatives and HPLC of desulfoglucosinolates. In another embodiment, the total glucosinolate content of the seed is no greater than 65 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 60 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 55 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 50 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 45 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed. In another embodiment, the total glucosinolate content of the seed is no greater than 40 μmol per gram of seed as determined by Near Infrared Spectroscopy (NIR) analysis of harvested seed.

Plant Breeding Technologies

[0120] Critical to the development and breeding of any crop is the ability to make use of genotypic and phenotypic diversity. Breeding strategies make use of the plant's method of pollination: self-pollination, where the pollen from one flower is transferred to the same or another flower on the same plant or a genetically identical plant; sib-pollination, when individuals with the same family or line are used for pollination; or cross-pollination, where the pollen comes from a flower on a genetically different plant from a different family or line.

[0121] For practical application, a breeder initially selects and crosses two or more parental lines, followed by repeated selfing and selection to produce many unique genetic combinations. For a newly developing crop such as carinata, it is necessary to be able to obtain a sufficient pool of genetic material to identify genetic backgrounds more adapted to target geographies (i.e. a better starting point), as well as variation for traits of interest. This allows for crossing or other modifications to be done to identify genetic combinations superior to the types already tested. Thus, an important initial objective is the collection and characterization of as large of a collection of genetic backgrounds as possible, for each target geography. Parental lines are selected based on breeding priorities and the unique combination of traits available in potential crossing parents. The selection of parents of these crosses is critical to the effectiveness of a breeding program. Parental lines may be closely or distantly related lines of a single plant species or may be two different species of the same genus.

[0122] Crossing, of (inbred) parental lines, by sexual hybridization, is typically done manually in controlled conditions. Often, two or three rounds of crossing are needed to accumulate beneficial alleles into a single genetic background. This includes evaluating offspring of a cross, selecting the most desirable (inbred) lines as future parents, and making the next round of parental selection based on priority targets. Theoretically, billions of different genetic combinations can be produced through a combination of mutagenesis, selfing, and crossing. Since the breeder has no direct control at the cellular level, two breeders will never independently develop the same variety of carinata plants have the same traits.

[0123] In each cycle of selection and evaluation, the breeder selects germplasm to advance to the next generation by growing individual plants in the chosen geography, soil and climate

conditions, and collecting phenotypic data reflective of actual performance that would be realized by a seed producer. Traits collected focus on those that would be of agronomic or economic benefit in the crop. Examples of traits characterized in a carinata breeding program include, but are not limited to, early plant vigor, plant height, branching habit, days to flower, silique density, flower petal color, pod size, reaction to heat and water stress, disease susceptibility, and pod shatter tolerance.

[0124] In a typical carinata breeding program, the breeder initially selects and crosses two or more parental *Brassica carinata* lines, followed by repeated selfing and selection to produce many unique genetic combinations, which are evaluated for overall agronomic potential as well as specific traits. Such recurrent selection can be used to improve a population of either self- or cross-pollinating *Brassica*. Intercrossing of several parents creates a genetically variable population of heterozygous individuals and the best plants are selected based on individual superiority, outstanding progeny and/or excellent combining ability. New populations are created by further intercrossing and selection. This method is useful for the improvement of quantitatively inherited traits controlled by numerous genes.

[0125] Backcrossing may be used to transfer genes for a simply inherited, highly heritable trait from a donor parent to the recurrent parent. After the initial cross, individual plants with the desired trait of the donor parent are selected and backcrossed to the recurrent parent for several generations. The resulting progeny are expected to have the attributes of the recurrent parent and the desired trait from the donor parent. Backcrossing may be used in conjunction with pedigree breeding. Pedigree breeding and recurrent selection methods are used to develop varieties from breeding populations. Pedigree breeding starts with the crossing of two genotypes, each with one or more desirable characteristics that is lacking in the other or that complement each other. Additional genotypes can be included in the breeding population if the original parents do not provide all the desired characteristics. In the pedigree method of breeding, five or more generations of selfing and selection may be used. For example, crossing of the two initial parents (the donor and recurrent parents) produces an initial F1 population, from which an F2 population is produced by selfing one or several F1 plants or by intercrossing two F1 plants (sib mating). Selection of hybrids with desired combination of traits may be conducted with the F2 population, or with the F3 or subsequent population.

[0126] Plants that have been self-pollinated and selected for several generations become homozygous at nearly all gene loci and produce a uniform population of breeding progeny or inbred lines. Subsequent crosses with two different homozygous (inbred) lines produces a uniform population of hybrid plants that may be heterozygous for many gene loci. Crossing two plants each heterozygous at a number of gene loci will produce a population that differ genetically and will not be uniform.

[0127] Doubled haploid (or DH) technology allows for the generation of completely homozygous lines, which are a combination of genes of the parental lines, in a single generation. This accelerates the process of inbred line development dramatically as the process from seeding of parental lines to obtaining seed for a resulting inbred population from those parents will generally take about 18 months. To achieve a highly homozygous line using traditional self-pollination generally will take five or six growth cycles, which in the case of *carinata* would represent three years or six growth cycles, with two cycles completed per year. In DH technology, using appropriate *in vitro* conditions, haploid microspores from an F1 plant can be induced to differentiate into diploid embryos and subsequently plantlets. This technique typically relies on a percentage of regenerated plants to undergo spontaneous doubling (usually in the range of 20 to 60% of plants, depending on several factors), whereas the remaining plants will remain haploid and sterile. To increase the efficiency of space used for seed increase, such as in the greenhouse or field, a flow cytometer is used to distinguish at an early stage the chromosome content “n” or “2n” of each plant. Thus, the sterile plants can be discarded at an early stage.

[0128] In some instances, a desirable trait may not reside within the species of interest, specifically *Brassica carinata*. In that case, it may be possible to transfer the trait via interspecific or wide crossing. For interspecific crossing, one parent is plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions and the second parent may be a different species of *Brassica*, including but not limited to *B. juncea* (L.) Czern. (brown mustard), *B. napus* L. (rape, Argentine canola), *B. nigra* (L.) W.D.J. Koch (black mustard), *B. rapa* L. (field mustard, Polish canola), and *B. oleracea* L. (cabbage, broccoli, cauliflower, brussels sprouts, kohlrabi and kale). In other instances, the other

Brassicaceae species including but not limited to *Brassica alba*, *Brassica hirta*, *Brassica juncea*, *Brassica napus*, *Brassica nigra*, *Brassica oleracea*, *Brassica rapa*, *Sinapis alba*, and *Camelina sativa*.

[0129] The methodology for performing interspecific crosses is similar to that described for within-species crosses described above. However, unlike intraspecific crosses, the likelihood that the resulting progeny will produce viable seed is very low and thus represents a formidable challenge to the success of this technique. To overcome this potential block, embryo rescue techniques are often employed to recover viable offspring from the cross. Essentially, this relies on the progeny surviving until the embryogenic stage at which point it can be dissected from the silique and placed into artificial growth medium. Under appropriate conditions, the cultured embryo can survive and be induced to differentiate into a plantlet, which can be grown into a mature plant. Successive rounds of embryo rescue may be needed until inbred progeny, or backcross-derived progeny, are stable and can produce fertile offspring without intervention. Often molecular markers, where available, are used to trace a specific allele from a related species into an adapted background in the target species, using repeated cycles of backcrossing.

[0130] In addition, to minimize the relative proportion of the donor genome from the non-carinata species, several rounds of back-crossing of the rescued plants with the *Brassica carinata* parent may be required to generate progeny having carinata character and the desired trait, which are stable and can produce fertile offspring.

Selection

[0131] Breeding nurseries are often the first cycle of evaluation of breeding populations. Generally, nurseries utilize single or paired rows with frequent checks (*i.e.*, the best available commercial germplasm for a specified geography).

[0132] Various methods are used to screen breeding populations for individual plants with desired characteristics. Single seed descent procedure refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has advanced from the F₂ to the desired level of inbreeding, the plants from which lines are derived will each trace to different F₂ individuals.

Since the number of plants in a population declines each generation due to failure of some seeds to germinate or produce seed, not all the F2 plants will be represented by a progeny in the final generation.

[0133] A multiple-seed procedure is when pods from each plant in a population are harvested and threshed together to form a bulk. Part of the bulk is used to plant the next generation and part is put in reserve. The advantage of this method is that it is faster to harvest seed with a machine than to remove one seed from each plant by hand. If desired, Double(d) Haploid methods can be used to extract homogeneous (homozygous) lines.

Marker Assisted Selection and Marker Assisted Breeding

[0134] A breeding program can make use of marker assisted selection (MAS) and marker assisted breeding (MAB) technologies to accelerate the successful outcome of a breeding project. These techniques enable the identification of lines carrying a trait of interest in the laboratory, while other lines not containing a marker of interest can then be discarded at an early stage. These methods can also increase the efficiency of a program, as the lines being evaluated in the field have a greater probability of meeting seed quality or other criteria. MAS and MAB methods rely on the existence of a dense set of genetic markers for the species of interest. Genetic markers are the unique sequences that may be found in allelic forms of genes, distinguishing one allele from another. Like genes themselves, they can be transmitted to progeny in a Mendelian fashion and can thus be used to follow the movement of specific alleles from parents to progeny.

[0135] Molecular markers can be used in Quantitative Trait Loci (QTL) mapping whereby selection of plants with desired trait(s) is assisted by markers known to be closely linked to alleles that have measurable effects on a quantitative trait—*i.e.*, accumulation of markers linked to positive effecting alleles or elimination of markers linked to negative effecting alleles in the plant genome. Markers can also be used to select for the genome of the recurrent parent and against the markers of the donor parent. This can minimize the amount of genome from a donor parent that remains in the selected plants and/or the number of back crosses to the recurrent parent.

[0136] Other types of genetic markers in common use by persons skilled in the art include, but are not limited to, Restriction fragment length polymorphisms (RFLP), Random Amplified

Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs).

Mutagenesis

[0137] Another method of creating genetic variation and capturing beneficial changes in a heritable fashion is through mutagenesis breeding. This method is often carried out via chemical means or ionizing radiation and is typically focused either on a microspore or on a whole seed level. In *Brassica* breeding, some common forms of mutagens used have been chemical agents such as ethyl methanesulfonate (EMS) or N-ethyl-N-nitrosourea (ENU), or high levels of ionizing radiation (x-ray or gamma irradiation or exposure to UV light). EMS produces random point mutations via low frequency methylation of guanine residues in genomic DNA. This results in altered Watson-Crick base pairing such that the affected base pairing is converted from G-C to A-T. ENU is also an alkylating agent that preferentially modifies thymine residues converting A-T to G-C. Ionizing radiation may affect DNA in many ways but typically the mutations are double strand breaks leading to deletions and frameshift mutations that are frequently inactivating.

[0138] To carry out this technique, seedlings or microspores are exposed to the mutagenic agent and the surviving fraction are allowed to develop into mature plants. In some cases, the mutagenized plantlets or embryos (in the case of microspore mutagenesis) may be exposed to selection in order to enrich for a particular phenotype. For example, mutagenesis has been used to develop plants that are resistant to the actions of specific herbicides; in this instance the developing plantlets or microspores can be grown *in vitro* in the presence of the herbicide(s) of interest in order to select for those plants with the appropriate mutations conferring resistance. The advantage of the microspore mutagenesis of the seed approach is that the resultant DH plants can be used to derive pure and homozygous plant lines where all induced mutations, whether dominant or recessive, would be expressed. Mutagenesis has been used to develop *Brassica* varieties with resistance to various herbicides, altered seed oil profiles and increased tolerance to disease and abiotic stress.

Genetic Transformation and Transgenic Technologies

[0139] In instances where unique and valuable traits are known to be available in distant plant or in non-plant species that cannot be transferred to *Brassica carinata* via classical breeding, and where the genes for those traits have been cloned, a breeding program may employ genetic transformation techniques, or transgenic technologies, to stably transfer those genes into this species. Transfer of cloned genetic elements into *B. carinata* have been achieved via a number of means, including PEG-mediated nucleic acid uptake into protoplasts (Johnson, *et al.*, 1989), electroporation into protoplasts (Fromm, *et al.*, 1985), ballistic infiltration using nucleic acid coated microprojectiles (Finer, *et al.*, 1999), *Agrobacterium*-based vector infiltration (Babic, *et al.*, 1998), and infection using plant virus-based vectors (Gleba, *et al.*, 2004). Aside from having the genes of interest in cloned form, the other requirements include having the genes cloned into a suitable vector to allow for their propagation in an appropriate bacterial system, as well as their packaging in appropriate viral and *Agrobacterium* strains if transformation utilizes an infectious route of transfer. Once transferred, the gene(s) of interest would also require appropriate plant-based promoters, enhancers and terminators to allow for the correct temporal and tissue specific pattern of expression for the heterologous gene. Finally, to select for those rare events where the heterologous gene expression unit has been successfully transferred into the plant genome, a selectable marker may be introduced, either physically linked to the heterologous gene of interest or co-transformed with the gene of interest at a suitable ratio to favor co-insertion.

[0140] The selectable marker may consist of a gene that can confer resistance to a particular herbicide or antibiotic that would otherwise kill the plant, a gene that may confer a growth advantage, a gene that may alter a response to plant hormones, or a gene that expresses a fluorescent protein that can allow transformed cells to be easily visualized. Examples of selectable markers conferring resistance to antibiotics that have been successfully used in *Brassica* transformation are the NPTII gene (Bevan 1984; Datla, *et al.*, 1992), encoding an enzyme conferring resistance to the antibiotic kanamycin, and the HPT gene encoding an enzyme conferring resistance to the antibiotic Hygromycin (Rothstein, *et al.*, 1987). Examples of selection markers based on conferring tolerance to herbicides and successfully used in *Brassica* transformation are the BAR (Thompson, *et al.*, 1987) and PAT (Wohlleben, *et al.*, 1988) gene products, which encode phosphinothicic acetyltransferase and confer resistance to glufosinate (bialaphos) or L-PPT, and the AHAS gene product encoding acetohydroxyacid

synthase enzyme conferring resistance to imidazolinones (Miki, *et al.*, 1990). Other plant selectable markers have been developed whose actions are not based on conferring resistance to toxic compounds per se but instead allow survival in the presence of nutrients not normally metabolized by the wildtype organism.

[0141] Transformation cannot only be used to introduce heterologous genes into the genome of *carinata* plants, it can also be used to introduce nucleic acid constructs that are designed to modulate the expression of endogenous genes. Genes encoding antisense RNA or RNAi sequences (Tang and Galili, 2004) can be used to interfere or knock down the expression of endogenous genes to extremely low levels, simulating the effect of a null mutation at the endogenous locus. This relies on the continuous stable expression of the antisense RNA or RNAi to be effective. In amphidiploid *Brassica* species such as *napus*, *juncea* and *carinata*, multiple copies of genes from the contributing ancestral species may create a high level of functional redundancy such that a single mutation in one of the homologues may not be sufficient to confer a phenotype. However, by using an RNAi or antisense approach, where the interfering RNA is derived from conserved sequences, one may conceivably be capable of targeting all the expressed homologues and achieving a functional knockdown effect.

[0142] Examples modifications that can be introduced into *Brassica carinata* using genetic transformation include, but are not limited to,

- genes that control pollination, hybrid seed production, or male-sterility;
- genes encoding resistance to pathogens and insect pests (plant disease resistance gene(s); gene(s) conferring resistance to fungal pathogens; natural or synthetic *Bacillus thuringiensis* (Bt) protein or a derivative thereof; an insect-specific hormone or pheromone coach or a mimetic based thereon, or an antagonist or agonist thereof; an insect-specific peptide that disrupts the physiology of the affected pest; an enzyme responsible for hyperaccumulation of a non-protein molecule with insecticidal activity; a membrane permease, a channel former or a channel blocker; a viral-invasive protein or a complex toxin derived therefrom; an insect-specific antibody or an immunotoxin derived therefrom; genes involved in the Systemic Acquired Resistance (SAR) Response and/or the pathogenesis related genes; antifungal genes; detoxification genes, such as for fumonisin, beauvericin, moniliformin, and zearalenone, and their structurally related

derivatives; cystatin and cysteine protease inhibitors; Defensin genes; genes that confer resistance to Phytophthora Root Rot, such as the *Brassica* equivalents of the Rps 1, Rps 1-a, Rps 1-b, Rps 1-c, Rps 1-d, Rps 1-e, Rps 1-k, Rps 2, Rps 3-a, Rps 3-b, Rps 3-c, Rps 4, Rps 5, Rps 6, Rps 7, and other Rps genes);

- genes that confer resistance to a herbicide (mutant ALS and AHAS enzymes to confer resistance to imidazolinone or sulfonylurea herbicides; mutant 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) and *aroA* genes conferring resistance to glyphosate; phosphinothricin acetyl transferase (PAT) and *Streptomyces hydroscopicus* phosphinothricin-acetyl transferase (BAR) conferring resistance to other phosphono compounds such as glufosinate; ACCase inhibitor-encoding genes conferring resistance to pyridinoxy or phenoxy propionic acids and cyclohexones; *pabA* and *gs+* genes conferring resistance to triazine; nitrilase gene conferring resistance to benzonitrile; acetohydroxy acid synthase, which has been found to make plants resistant to multiple types of herbicide);
- genes that confer or contribute to altered fatty acids (down-regulation of stearoyl-ACP desaturase to increase stearic acid; elevating oleic acid via FAD-2 gene modification and/or decreasing linolenic acid via FAD-2 gene modification; altering conjugated linolenic or linoleic acid content; altered LEC1, AGP, Dek1, Superal1, *mil* ps, various *lpa* genes such as *lpa 1*, *lpa3*, *hpt*, or *hggt*);
- genes that confer or contribute to altered phosphorus content (introduction of a phytase-encoding gene; up-regulation of a gene that reduces phytate content);
- genes that confer or contribute to altered carbohydrates, antioxidant, or essential seed amino acids;
- genes that create a site for site specific DNA integration, such as the introduction of FRT sites that may be used in the FLP/PRT system and/or Lox sites that may be used in the Cre/Loxp system;
- genes that affect abiotic stress resistances (including, but not limited to flowering, pod and seed development, enhancement of nitrogen utilization efficiency, altered nitrogen

responsiveness, drought resistance or tolerance, cold resistance or tolerance, and salt resistance or tolerance, and increase yield under stress).

Gene Editing

[0143] More recently, several novel approaches have been developed which offer the ability to manipulate the plant genome in a targeted way. Collectively known as gene editing technology (Petolino, *et al.*, 2010; Woo, *et al.*, 2015, Sauer, z *et al.*, 2016), the technologies share several important similarities:

- they offer the ability to introduce small or large gene deletions or insertions in the plant genome by means of targeted double strand breaks at precise genomic locations;
- they allow for the insertion of small inactivating mutations into specific genes of interest;
- they permit the replacement of an entire gene or gene segment by a modified counterpart;
- they allow for the insertion of a heterologous gene in a specific genomic location which may represent a preferred site for regulated gene expression. (i.e. downstream of a known endogenous promoter/enhancer).

Locus Conversion

[0144] The *Brassica carinata* plants described herein represents a base genetic line with a novel trait (a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions) into which other new loci or traits may be introduced using transgenic technologies or back-crossing. The term “locus conversion” refers to the product of such an introgression. For example, a donor parent having a specific desirable trait may be crossed with an inbred variety, the recurrent parent, which has overall good agronomic characteristics yet lacks the desirable trait. The progeny of this cross is then mated back to the recurrent parent followed by selection of the desired trait from the donor parent.

[0145] A plant cell of a locus converted plant is obtained by introducing one or more locus conversion in the *Brassica carinata* plant described herein. The locus converted plant cell is therefore identical to a cell from the *Brassica carinata* plant described herein except for the one

or more locus conversion and the locus converted plant exhibits essentially all the physiological and morphological characteristics of the *Brassica carinata* plant described herein. The locus conversion can confer a trait selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism. Both naturally-occurring and transgenic nucleic acid sequences may be introduced through backcrossing. Molecular marker assisted breeding or selection may be used to reduce the number of backcrosses need to achieve the backcross conversion.

[0146] A locus conversion of the *Brassica carinata* plant described herein will otherwise retain its genetic integrity. For example, a locus converted *Brassica carinata* plant described herein will comprise at least 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of its base genetics of the starting *Brassica carinata* plant. A locus converted *Brassica carinata* plant described herein will therefore have at least one, and possibly two or three physiological or morphological characteristics which are different from those of a locus converted *Brassica carinata* plant described herein and otherwise has all the physiological or morphological characteristics of a locus converted *Brassica carinata* plant described herein.

Male sterility

[0147] To produce and test hybrid combinations, some form of pollination control system must be employed. The most widely used pollination control system to achieve this in *Brassica* species is the method of Ogura (Ogura, 1968) adapted and modified by scientists at INRA, which is based on control of pollination by use of male cytoplasmic sterility. In cytoplasmic male sterile (CMS) plants, a mitochondrial gene mutation interferes with the flower's ability to produce viable pollen but does not affect the functionality of the flower's female components. Because the mutation is within the mitochondrial genome, it is transmitted maternally through the cytoplasm. CMS plant lines, termed A lines, can only produce viable seed via outcrossing with non-CMS plants; however, the F1 plants derived from the seed of such a crossing will also exhibit the CMS phenotype and will still not produce viable seed by self pollination.

[0148] The Ogura CMS trait, originally identified in Japanese radish varieties (Ogura, 1968), was transferred to *Brassica oleracea* via backcrossing (Bannerot, *et al.*, 1977) then subsequently transferred to *Brassica napus*. These initial *Brassica napus* A lines were found to be deficient in terms of their sensitivity to cold and their tendency to exhibit leaf chlorosis, as well as fertility

issues, which were ultimately found to be related to interactions with chloroplasts derived from the radish cytoplasm (Pelletier, Primard *et al.*, 1983). Using a cell fusion approach, improved *Brassica napus* A lines were subsequently developed that overcame these Initial deficiencies (Pelletier, *et al.*, 1987). A lines can be maintained by crossing them with B lines (lines that are genetically identical to A lines except that their cytoplasm is not CMS).

[0149] For production of fully fertile hybrid seed, CMS lines must be crossed with lines that contain a restorer function gene (Rf) which can restore the ability of CMS plants to produce viable pollen. Plant lines with the RF function stably incorporated and homozygous (termed RF lines) can be crossed with CMS lines and the F1 seed harvested from the CMS plants will represent a true hybrid and which will have the ability to produce fully fertile offspring capable of self pollination, a prerequisite for commercial grain production.

[0150] Such an Rf activity, first identified in radish, was introduced into *Brassica napus* (Heyn, 1976). It was subsequently shown that these lines were affected by fertility problems (Heyn, 1978; Pellan-Delourme and Renard, 1988). Intensive backcrossing of these initial lines eventually yielded material with improved fertility such that they could be used practically as Rf lines (Delourme, *et al.*, 1991). It was further noted, however, that hybrid seed produced from crosses with the early versions of *Brassica napus* Rf lines often had high glucosinolate levels (Delourme, Foisset *et al.*, 1998) that made generation of canola quality hybrids problematic. Efforts to develop improved Rf lines by reducing the amount of extraneous radish genetic material has resulted in newer generations of *Brassica napus* (Primard-Brisset, *et al.*, 2005) and *Brassica juncea* (Tian, *et al.*, 2014) Rf line families of sufficiently quality to be used in commercial hybrid seed production. Seed of Rf lines can be maintained by allowing self crossing of plants grown in isolated tents or fields.

[0151] A, B and Rf lines are thus the prerequisite components of an expandable production system for F1 hybrid seed. In practice, for production of pure hybrid seed:

- a. the hybrid production fields are required to be reproductively isolated from extraneous sources of *Brassica* pollen;
- b. Rf lines are seeded at a predetermined optimal ratio relative to A lines and may be kept spatially separated from the A lines so that the Rf plants can be readily removed post flowering, and prior to harvest of the A line material;

- c. pollinators are absolutely required to allow for maximal pollination of the A lines by CMS pollen donors;
- d. for maintenance and expansion of Rf stocks, Rf seed is planted in isolation (either isolated fields separated from other *Brassica* crops by great distance, or plots that are reproductively isolated by virtue of a physical barrier such as a tent) and allowed to self pollinate;
- e. for maintenance and expansion of A line stocks, A lines and B lines are planted in separate rows in isolated fields or tents (as described above) and pollinators are used to promote pollination of A lines with B line pollen. B lines are cut down after flowering and before seed set and the A line plants are allowed to come to maturity then harvested.

In some cases, a less labor- and cost-intensive alternative to that described in (b) is employed whereby a low ratio of Rf plants may be interspersed with the A line plants in the field and harvested together. While there will be some level of adulteration of the harvested hybrid grain with that of the self pollinated Rf plants, however, in practice this level of contamination will not greatly affect the commercial quality of the hybrid seed

[0152] More recently, both the CMS and Rf traits have been transferred to *Brassica carinata*. The development of a set of genetically diverse CMS and Rf lines in *Brassica carinata* allows the testing of many combinations for the first time in this *Brassica* species.

[0153] In a hypothetical test scheme based on the Ogura CMS system, the following steps are carried out:

- panels of genetically diverse *Brassica carinata* plants are selected from a previously characterized collection of elite germplasm and used for introgression of CMS or Rf traits to produce A and Rf test lines. Diversity can be estimated by comparing differences in molecular marker profiles between candidate lines;
- crosses are carried out between selected diverse A and Rf test lines, under conditions described in previous sections;
- F1 *carinata* hybrid seed from test crosses are planted in replicated small plot trials, F1 plants and harvested seed are assessed for a number of traits including, but not limited to, early vigour, yield potential, grain and oil yield per acre, as well as seed quality traits;

- patterns of heterosis between pairs A and Rf parents are identified and F1 hybrid seed from promising combinations are studied more extensively in larger, geographically diverse trials; and
- hybrid seed from specific combinations may be selected for potential commercial release.

Phenotype/Genotype

[0154] To be useful and reliable, a *Brassica carinata* variety or hybrid must be homogenous and reproducible. While there are a number of analytical methods known to the skilled person for assessing the phenotypical stability of a *Brassica carinata* variety or hybrid, the traditional method is the observation of phenotypic traits over the life of the carinata plant using data collected from field experiments conducted under the selected geographic, climatic, and soil conditions during one or more growing seasons. Phenotypic characteristics observed include, but are not limited to, traits associated with seed yield (pod density, number of seeds per pod, pod length), oil yield, seed oil quality (GSL and erucic acid content; fatty acid composition), seed protein content, seed protein quality, glucosinolate composition of meal, growth habit, lodging resistance, plant height, and pod shatter resistance. Other phenotypic characteristics that may be observed include, but are not limited to, traits associated with pest tolerance or resistance, cold or frost tolerance, disease tolerance or resistance, herbicide tolerance or resistance, early or late flowering, and/or early or late maturity.

[0155] In some embodiments, *Brassica carinata* varieties or hybrids useful for commercial crop production or production of commercial products may exhibit traits associated with seed yield, oil yield, seed oil quality, erucic acid content of seed or oil, glucosinolate content of seed, seed protein content, fatty acid composition of oil, glucosinolate composition of meal, meal protein content, growth habit, lodging resistance, plant height, and pod shatter resistance

[0156] In some embodiments, a *Brassica carinata* variety or hybrid may exhibit multiple traits or phenotypic characteristics that provide agronomic advantages in particular geographies, climates, cropping regimes, and/or soil types. For example,

- *Brassica carinata* varieties for planting in regions with a climate classified as being of tropical moist characteristics, with planting occurring in fall or winter for harvest in spring or summer, may be selected for one or more traits including, but not limited

to, superior yield of oil per area planted, shorter time to maturity, improved frost tolerance, improved disease resistance, and resistance to pod shatter.

- *Brassica carinata* varieties for planting in regions with a climate classified as being of cool temperate, dry classification, with planting occurring in spring and harvest in summer or fall, may be selected for one or more traits including, but not limited to, superior yield of oil per area planted, shorter time to maturity, tolerance to drought, improved disease resistance, and resistance to pod shatter.
- *Brassica carinata* varieties for planting in regions with a climate classified as being of warm temperate, moist characteristic for planting in fall or winter for harvest in spring or summer, may be selected for one or more traits including, but not limited to, superior yield of oil per area planted, shorter time to maturity, tolerance to drought, improved disease resistance, and resistance to pod shatter.

[0157] Other traits for which *Brassica carinata* varieties may be selected include, but are not limited to, *Alternaria* resistance, days to flowering, depth of canopy, duration of flowering, end of flowering, flower petal coloration, frost tolerance, herbicide resistance, leaf colour, leaf glaucosity, leaf length, leaf number of lobes, leaf width, maturity, seed maturity, number of seed-bearing pods, pod (silique) beak length, pedicel length, petiole length, plant height, plant length, pod (silique) length, pod (silique) width, primary raceme length, recovery from frost damage, *Sclerotinia* resistance, seed colour, seeds per pod, seed weight (thousand seed weight or TKW), and stem color.

[0158] Genotype assessment(s) can be used to confirm the homogeneity and reproducibility of a *Brassica carinata* hybrid, to identify plants of the same variety or related variety, and to confirm the pedigree of the plant. Techniques known to those skilled in the art for the analysis and comparison of plant genotype include, but are not limited to, whole genome sequencing, Restriction fragment length polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs).

[0159] The *Brassica carinata* plants of the present invention have shown uniformity and stability for all traits described in the variety description information in Tables 1, 2, 6, 7, and 11 which include morphological, agronomic, and quality traits for *Brassica carinata* plants carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions, such as *Brassica carinata* DH-146.047, DH-146.194, DH-146.214, or DH-146.842. The detailed phenotypic information provided in Tables 1, 2, 6, 7, and 11 is based on data collected in field experiments using conventional agronomic practices. For comparative purposes, *Brassica carinata* AAC-A120 and AGR044-312D-HP11 (WO2017/181276A1; henceforth referred to as “AGR044-HP11” or “HP11”) were similarly grown as comparative check varieties in replicated experiments, and observations were recorded for the various morphological traits for *Brassica carinata* DH-146.047, DH-146.194, DH-146.214, or DH-146.842 and the comparative check varieties.

Disease

[0160] Varieties of *Brassica carinata* may be susceptible to a number of pathogens that commonly infect *Brassica species* including, but not limited to, the bacteria and fungi listed below:

Disease	Organism	Disease	Organism
Bacterial leaf spot	<i>Pseudomonas syringae</i>	Light leaf spot	<i>Pyrenopeziza brassicae</i>
Bacterial leaf rot	<i>Erwinia marginalis</i>	Powdery mildew	<i>Erysiphe polygoni</i>
Bacterial soft rot	<i>Pseudomonas marginalis</i>	Root rot complex	<i>Rhizoctonia solani</i> , <i>Fusarium spp.</i> , <i>Pythium spp.</i>
Black rot	<i>Xanthomonas campestris</i>	Seeding disease complex	<i>Rhizoctonia solani</i> , <i>Fusarium spp.</i> , <i>Pythium spp.</i>
Alternaria black spot	<i>Alternaria spp</i>	Sclerotonia white stem rot	<i>Sclerotonia sclerotiorum</i>
Alternaria grey leaf	<i>Alternaria brassicae</i>	Aster yellow	<i>Phytoplasma spp.</i>

<i>spot</i>	<i>Alternaria brassicola</i>		
<i>Alternaria pos spot</i>	<i>Alternaria brassicae</i> <i>Alternaria brassicola</i>	<i>Damping off</i>	<i>Phytophthora cactorum</i> <i>Pythium</i> spp.
<i>Black leg</i>	<i>Leptosphaeria maculans</i> <i>Leptosphaeria biglobosa</i>	<i>White leaf spot</i>	<i>Mycosphaerella capsellae</i>
<i>Black root</i>	<i>Aphanomyces raphanin</i>	<i>Grey stem</i>	<i>Mycosphaerella capsellae</i>
<i>Clubroot</i>	<i>Plasmodiophora brassicae</i>	<i>Wirestem and girdling root rot</i>	<i>Rhizoctonia solani</i>
<i>Downey mildew</i>	<i>Peronospora parasitica</i>	<i>White rust</i>	<i>Albugo candida</i>
<i>Fusarium wilt</i>	<i>Fusarium oxysporum</i> <i>Fusarium avenaceum</i>	<i>Verticillium wilt</i>	<i>Verticillium longisporum</i>

[0161] Examples of economically significant fungal diseases of *Brassica* species and mustard oilseeds include

- a. ***Sclerotinia* stem rot** is caused by a fungus whose spores infect *Brassica* species primarily during flowering stages and whose incidence is associated with periods of high humidity. Lesions are formed on the stems which can eventually kill the plant. Fungicides are available which can control the severity of the infection but must be applied at specific periods of the plant lifecycle (i.e. at early to mid-flowering) for best effect. Often multiple applications within this window of time are necessary.
- b. ***Alternaria*** is a fungal disease of *Brassica* species that affects plants at all growth stages from early seedling through to maturity although mature plants are more susceptible. The greatest economic impact is on grain yield and quality. Foliar fungicide application during the late flowering stage is an effective way to mitigate the more detrimental effects of the disease on grain yield and quality.
- c. **Blackleg** is caused by a fungal pathogen *Leptosphaeria maculans* of *Brassica* oilseed crops, which infects plants at all stages, but early stage infections have the most serious consequences, often culminating in plants with necrotic lesions on their lower stems that

can virtually sever the plants at the base. Fungicides are only partially effective, having a minor protective effect when applied at an early plant growth stage.

- d. **Clubroot** is caused by a soil borne fungus-like pathogen (*Plasmodiophora brassicae*) that affects the roots of *Brassica* oilseed crops. The spores can persist for long periods in the soil and there is currently no effective fungicidal treatment. Management may require using rotations which limit the frequency of *Brassica* planting.

[0162] The development of disease tolerant or disease resistant carinata varieties is important for achieving high yields of seed, oil, and other products from *Brassica carinata*. Conventional methods for control of microbial disease may employ one or more of chemical control, disease resistance, and culture control procedures such as crop rotation, liming, and use of bait crops.

[0163] When producing a commercial crop or a group of cultivated *Brassica carinata* plants in a field, harmful microorganisms can be controlled by the application of a composition comprising one or more microbiocidal ingredients, such as a fungicide. Fungicides comprise a diverse set of chemical agents which can prevent or reduce the severity of plant infection by pathogenic fungi. There are numerous classes of fungicides. FRAC (Fungicide Resistance Action Committee; <http://www.frac.info/home>) lists 12 classes based on the different biochemical pathways that the fungicides within a class targets, as well as a 13th class which comprises fungicides with unknown modes of action. Fungicides are also distinguished by their modes of delivery and sites of action: some fungicides are sprayed onto the plant's surfaces, some are applied to the soil surfaces either in granular form or as a liquid flooding the soil surface, while others are applied as seed treatments. Fungicides applied to the seeds or soils tend to be absorbed via the roots and are transported to all plant tissues via xylem. Fungicides that are foliar can be either local—*i.e.*, protecting only the surfaces that they contact, systemic—*i.e.*, absorbed by the upper plant surfaces but then transported by xylem to all above ground tissues, or partially systemic—*i.e.*, they can be locally absorbed but can only be transported short distances to protect a somewhat more extensive surface than the initial point of fungicide contact. Fungicides can help mitigate the risk of losses incurred by fungal infection, but the costs of fungicide spraying are significant enough to require cost benefit and risk assessment type analyses to be carried out before deciding to proceed.

[0164] When producing a commercial crop or a group of cultivated *Brassica carinata* plants in a field, harmful microorganisms can be controlled by the application of a composition comprising one or more microbicide or fungicide ingredient. Examples of microbiocides and fungicides useful for disease control for *Brassica carinata* include, but are not limited to, azoxystrobin, boscalid, fluxapyroxad, pyraclostrobin, picoxystrobin, propiconazole, metconazole, iprodione, prothiaconazole, vinclozolin, carbathiin, thiram, difenoconazole, metalaxyl, sedaxane, fludioxonil, penflufen, trifloxystrobin, and sedaxane.

Insect Pests

[0165] A variety of insect pests may infest and/or cause damage to a developing *Brassica carinata* plant. Common insect pests of *Brassica* at the seedling stage include, but are not limited to, aphids (*Lipaphis erysimi*, *Myzus persicae*, *Brevicoryne brassicae*), flea beetles (*Phyllotreta cruciferae*, *Phyllotreta striolata*, *Psylliodes punctulata*), cutworm (*Agrotis orthogonia*, *Euxoa ochrogaster*, *Feltia jaculifera*, *Lacinipolia renigrea*), and cabbage root maggot (*Delia radicum*). Common insect pests of *Brassica* at the flowering and podding stages include, but are not limited to, diamondback moth (*Plutella xylostella*), Berta armyworm (*Mamestra configurata*), and cabbage seed pod weevil (*Ceutorhynchus obstrictus*).

[0166] When producing a commercial crop or a group of cultivated *Brassica carinata* plants in a field, insect pests may be controlled by the application of a composition comprising one or more insecticide. Insecticides are a group of pesticide compounds designed to reduce or eliminate crop loss due the predation of crop species by insects. Like herbicides and fungicides, insecticides are classified according to their mode of action and the biochemical pathways that they target. One classification scheme (IRAC MoA) advocated by the Insecticide Resistance Action Committee (IRAC; <http://www.irac-online.org>) lists 29 classes of insecticides grouped by the common biochemical processes and pathways that the insecticide compounds target. Like herbicides and fungicides, insecticide function and persistence can also be influenced by their sites of action, i.e. whether they are only active on the surface of plants as applied, or whether they function as systemic agents. Further differentiation among some insecticide groups may be apparent based on whether they exhibit selectivity for specific insect types due to distinctive aspects of that insect's biology. Given that some insects serve a beneficial role, such as

controlling plant pests, serving as plant pollinators and improving the nutrient content of soil, it is important that insecticides not be applied indiscriminately, but rather are used in a way that limits their actions as much as possible to the desired target species. Thus, modalities such as timing of application, amount and route of application, and restrictions on the types of insecticides used and the crops they may be used are all incorporated into the registered usage criteria of insecticide as a means of ensuring their safety and efficacy.

[0167] Insecticides useful for the control of insect pests of *Brassica carinata* include, but are not limited to, zeta-cypermethrin, zeta-cypermethrin-S-cyano, lambda-cyhalothrin, methoxyfenozide, cyantraniliprole, imidacloprid, thiamethoxam, and clothianidin. Such insecticide can be applied as a seed treatment or as a foliar treatment.

Plant pests

[0168] *Brassica carinata* will out-compete many weeds if it establishes well. Some weed species, however, if allowed to establish early and persist, can affect quality and yield of all crops, including *carinata*. Examples of weeds that can adversely affect yield and quality include cochia, wild mustard, and wild radish. Weed management is thus an important aspect of modern agricultural practice and comprises several different but complementary approaches including physical methods to remove weeds before seed can be set, such as cultivation, tilling and rogueing of fields as well as use of chemical agents or herbicides to suppress or kill weedy species before they become established and/or are able to set and release their seed.

[0169] When producing a commercial crop or a group of cultivated *Brassica carinata* plants in a field, plant pests can be controlled by the application of a composition comprising one or more herbicide. Herbicides comprise a large group of chemical compounds that interfere with specific biological processes of the plants in such a way as to block their growth and survival. Herbicides are grouped into classes defined by the biological process with which they interact. These can include inhibition of lipid biosynthesis, inhibition of amino acid biosynthesis, hormonal regulation of plant growth, inhibition of photosynthesis, inhibition of nitrogen metabolism, inhibition of plant pigments biosynthesis or function, agents which can disrupt cell membranes and agents which inhibit seedling growth (Sherwani, *et al.*, 2015). In general, different compounds and herbicide classes may display preferential efficacy against certain weedy species. Moreover, some crop species may display more tolerance to certain classes of herbicide

than others. Thus, in a particular geographical region, the use of a particular herbicide for weed control may be dictated by the nature of the crop being cultivated and the native weeds encountered in the region. The registered usage also specifies specific methods of application of the herbicide, including recommended concentration of herbicide, use of appropriate diluents, adjuvants, or surfactants, method of delivery (i.e. spray versus granular), timing of application at appropriate crop stage to ensure least crop damage, timing of application and number of applications to ensure optimal weed control, location of application (foliar or soil application), recommended weather conditions for optimal weed control. Some examples of herbicides recommended for use with *Brassica carinata* grown in SE USA are listed (Seepaul, *et al.*, 2015).

Seed cleaning

[0170] “Cleaning (of) seed” or “seed cleaning” refers to the removal of foreign material from the surface of the seed. Foreign material to be removed includes, but is not limited to, fungi, bacteria, insect material (including insect eggs, larvae, and parts thereof), and any other pests that exist on the surface of the seed. “Cleaning (of) seed” or “seed cleaning” also refers to removal of any debris or low quality, infested, or infected seeds, or seeds of different species that are foreign to the sample.

Seed treatment

[0171] Prior to planting, *Brassica carinata* a composition may be applied to the seed as a seed treatment. The composition may be applied at any time from harvesting of the seed to sowing of the seed using methods including, but not limited to, mixing in a container, mechanical application, tumbling, spraying, misting, and immersion. The composition may be applied as a liquid, a slurry, a mist, a soak, or a powder. The composition may comprise one or more of a pesticide, fungicide, insecticide, antimicrobial, a bacterial or fungal inoculant for nutrient utilization, plant growth regulator, or plant signalling compound. A general discussion of techniques for application of fungicides to seeds may be found, for example, in chapter 9 of (Jeffs 1978)

Commercial crops and commercial plant products

[0172] Commercial crop production comprises the steps of seeding, cultivation and harvesting of grain:

- **Seeding:** *Brassica carinata* can be planted into conventionally tilled soil where conventional tillage or full-tillage comprises a substantial soil inversion repeated several times yearly such that few plant residues remain at the soil surface at the time of seeding. Alternatively, *carinata* can be planted into soil that is maintained under conservation tillage practices whereby the extent and frequency of tillage is substantially reduced with respect to conventional tillage (so-called medium or low tillage soil management) or preferably, it may be no-till planted in standing stubble. Seeding is carried out at a rate designed to achieve plant densities in a range from 80 to 180 plants per square meter. *B. carinata* should be seeded at a consistent 1.25 to 2.5 cm depth. *Brassica carinata* is a mid- to long-season crop that requires a slightly longer growing season than other mustard types. Hence seeding early provides the best results. The ideal seeding date depends greatly on geography and weather. In general, soils should be at least 4°C (40°F) or higher before planting. In the Canadian Prairies and US northern tier, typical planting occurs in spring between early April to late May. In South Eastern US, typical planting occurs in fall between October and December. In South America, the optimal planting time occurs in fall or winter (i.e. typically between beginning of May to end of June).
- **Cultivation:** For good stand establishment, *Brassica carinata* requires adequate soil moisture at seeding and through emergence but can tolerate reduced moisture thereafter and stands up well to the semi-arid mid-summer conditions. *Brassica carinata* is a temperate climate crop but which has been adapted to the more extreme conditions experienced in the southern Canadian prairies and Northern Tier US states. During initial stand formation, *carinata* can recover from short term frost conditions and tolerates higher heat during flowering and seed set better than other *Brassica* oilseeds. The fertility requirements of *Brassica carinata* are similar to other mustards and canola. Adequate availability of the primary macronutrients nitrogen, phosphorous, potassium and sulfur are required to achieve the true yield potential. Lesser amounts of secondary macronutrients, including calcium (Ca), magnesium (Mg) and sulfur (S) and trace amounts of micronutrients (such as boron, copper, Iron, manganese, zinc) may also contribute to optimal plant growth and yield. Fertilizer rates vary with growing zone and soil fertility

- **Harvesting** is the act of collecting the portion of a plant that has matured sufficiently over the course of a growing season and that has value as a source of food, feed, fibre, feedstock, structural material or as a propagule for the plant itself. *Brassica carinata* is harvested, for example, by mechanical harvesting, ideally when seed maturity is reached (seed, pods and stalks turn from green to yellow, seed moisture is 9.5 5 or less). *Brassica carinata* can be combine harvested by straight cutting or, if need be can be swathed at an early stage, allowed to dry naturally or with the aid of a desiccant, then the dried swath can be combined. Swathing mean cutting near the base of the plant and allowing the plant to lie flat in field for several days to allow the grain to reach the appropriate dryness. However, since *Brassica carinata* has a sturdy stalk, the preferred method for harvest of carinata is direct combining at maturity, rather than swathing or pushing followed by combining.
- **Combining:** refers to the process of reaping and collecting the seed pods from the matured crop, threshing the seed pods to release the seed (grain), and winnowing to separate and recover the grain from the now empty seed pods, stems, and branches (collectively referred to as chaff). These once distinct operations are today often “combined” by use of a multifunctional mechanized apparatus, appropriately known as a “combine” harvester.
- **Grain**, in reference to *Brassica carinata*, refers to the seed harvested at maturity and sold as a source of oil and meal products.

[0173] Commercial products produced from *Brassica carinata* seed include, but are not limited to, crushed, non-viable seed, oil, meal, and protein isolate. Production of oil, meal and protein isolate involves multiple steps. Typically, the seeds are cleaned then crushed in a roller mill to generate flakes. The flaked seed then undergoes a cooking process in which it is conveyed to a heated drum where the flakes are cooked at elevated temperatures (typically from 70-90°C). The cooking helps to reduce the viscosity of the oil to allow for more efficient extraction in subsequent steps. Cooked seed flakes are then pressed in a series of screw presses or expellers which can remove 50-60% of the oil. Aside from the oil, which is removed for further processing, the pressing produces a meal cake that is ideal for solvent extraction. Using several cycles of extraction, the meal cake is treated with a solvent such as hexane to remove the residual

oil from the meal. The meal is then transferred to a desolventizer-toaster where it is heated to remove remaining solvent; the final step of the process, called toasting, involves injection of steam into the meal to remove the last traces of solvent. The meal is then cooled and dried by blowing forced air through it. In some cases, the seed can also be processed using a cold press methodology which is similar to above except it does not involve the use of solvent to remove residual oil from the oil cake, resulting in a meal with much higher oil composition. In other cases, meal containing high levels of glucosinolates can be combined with other ingredients and formulated into pellets for use as a biofumigant (U.S. Publication No. 2008/0199451).

[0174] *Brassica carinata* plants can also be used commercially for biofumigation to reduce the population of disease-causing organisms in the soil. Typically, a field is seeded with *Brassica* plants early in the planting season, the plants are grown for a time, and the biomass is collected between flowering and before seed set. The plant biomass is then chopped to release the myrosinase enzyme and convert the glucosinolates in the plant biomass to isothiocyanates. The chopped biomass is tilled into the soil prior to seeding of a second crop.

Uses of *Brassica carinata* plants producing seed with reduced glucosinolate (GSL) content

[0175] The *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions, can be used in accordance with any of the breeding methods described herein, as well as in breeding methods known to those skilled in the art, to produce *carinata* hybrids or other progeny plants having desired traits and characteristics of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* in the same location under the same environmental conditions as described herein.

[0176] The invention is directed to methods for producing *carinata* seeds, cells, plants, and plant parts from a *carinata* plant produced by crossing a first parent plant with a second parent plant, wherein the first parent plant is the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata*

grown in the same location under the same environmental conditions, described herein, and the second parent plant is the same *Brassica carinata* plant, a different *Brassica carinata* plant, or a plant of another *Brassicaceae* species. In some embodiments, the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, may be the male or female parent. In other embodiments, either the first or second parent plant may be male sterile. In some embodiments, the second parent plant comprises a desired trait. In some embodiments, the desired trait is selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism. In other embodiments, the desired trait is herbicide tolerance and the herbicide is selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba.

[0177] In another aspect, the invention is directed to a method of producing a first generation (F1) hybrid *Brassica carinata* seed, as well a first generation (F1) hybrid plant grown from such seed, comprising crossing the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, with a different *Brassica carinata* plant and harvesting the resultant F1 hybrid *Brassica carinata* seed, and wherein the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, is either a female parent or a male parent.

[0178] In another aspect, the invention is further directed to a method of for producing a Doubled Haploid (DH) comprising isolating a flower bud of a F1 hybrid plant, dissecting out a haploid microspore, placing the haploid microspore in culture, inducing the microspore to differentiate into an embryo and subsequently into a plantlet, identifying whether the plantlet contains a diploid chromosome number, wherein the diploid chromosome number occurred through chromosome doubling, and continuing to grow the plantlet if it contains a diploid

chromosome number. In one embodiment, the diploid chromosome number is obtained by chemical or physical means. In another aspect, the invention is directed to a plant, plant part, or seed of such Double Haploid produced from the F1 hybrid plant resulting from crossing the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, with a different *Brassica carinata* plant.

[0179] In another aspect, the invention is directed to producing a *Brassica carinata* plant, plant part, cell, or seed therefrom, by crossing the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, with a second *Brassica carinata* plant having a desired trait, growing the resultant F1 hybrid seed and selecting one or more progeny plants that have the desired trait, backcrossing the selected progeny plants that have the desired trait with plants of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, to produce backcross progeny seed, and growing the resultant backcross progeny seed and selecting backcross progeny plants that have the desired trait. In some embodiments, the steps of backcrossing and growing the resulting progeny seed may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and produces seed with a total glucosinolate content reduced by at least 14% relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In other embodiments, the steps of backcrossing and growing the resulting progeny seed may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica*

carinata plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and produces seed with a total glucosinolate content reduced by at least 8 μmol per gram of seed relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0180] In other embodiments, the steps of backcrossing and growing the resulting progeny seed may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5 6, 7, 8 , 9, 10, 11, 12, or 13, as determined at the 5% significance level. In some embodiments, the desired trait is selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism. In other embodiments, the desired trait is herbicide tolerance and the herbicide is selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba. In other embodiments, the present invention is also directed to a cell, seed, plant, or plant part of a *Brassica carinata* plant having a desired trait, as well as seeds, plants and plant parts thereof, produced by the breeding methods described above.

[0181] In yet another aspect, the invention is directed to a method of producing a *Brassica carinata* plant having a desired trait, as well as seeds, cells, and plant parts thereof, by crossing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, with a plant of another species of the family *Brassicaceae* comprising the desired trait, producing F1 progeny plants using embryo rescue techniques to recover viable F1 plants or growing F1 seeds, self-pollinating the F1 plants that

have the desired trait and carinata character, producing F2 plants using embryo rescue techniques to recover viable F2 plants or growing F2 seeds, self-pollinating the F2 plants that have the desired trait and carinata character, using embryo rescue techniques to recover viable F3 plants or growing F3 seeds to produce progeny plants, self-pollinating the progeny plants that have the desired trait and carinata character to produce further progeny plants, and selecting the progeny plants with the desired trait and carinata character. In some embodiments, the steps of producing progeny plants, self-pollinating, and selecting progeny plants having the desired trait and carinata character are repeated until the progeny plant has the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata*. In other embodiments, the steps of producing progeny plants, self-pollinating, and selecting progeny plants having the desired trait and carinata character may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and produces seed with a total glucosinolate content reduced by at least 14% relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In other embodiments, the steps of producing progeny plants, self-pollinating, and selecting progeny plants having the desired trait and carinata character may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and produces seed with a total glucosinolate content reduced by at least 8 μmol per gram of seed relative to the total

glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0182] In other embodiments, the steps of producing progeny plants, self-pollinating, and selecting progeny plants having the desired trait and carinata character may be repeated 1 to 2 times, 1 to 3 times, 1 to 4 times, or 1 to 5 times, 1 to 10 times, or until the *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, has the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, the desired trait is selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism. In other embodiments, the desired trait is herbicide tolerance and the herbicide is selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba. In other embodiments, the present invention is also directed to a cell, seed, plant, or plant part of a *Brassica carinata* plant having a desired trait, as well as seeds, plants and plant parts thereof, produced by the breeding methods described above.

[0183] Another aspect of the present invention is directed to a tissue culture of protoplasts or regenerable cells of a seed, plant or plant part produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein. The tissue may be selected from the group consisting of leaves, pollen, embryos, roots, root tips, pods, flowers, ovules, and stalks.

[0184] In some embodiments, the present invention relates to a *Brassica carinata* plant regenerated from the tissue culture having the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5 6, 7, 8 , 9, 10, 11, 12, or 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata*. In some embodiments, the *Brassica carinata* plant regenerated from the tissue culture produces seed with a total glucosinolate content reduced by at least 8 μmol per gram of seed relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions. In some embodiments, the *Brassica carinata* plant regenerated from the tissue culture produces seed with a total glucosinolate content reduced by at least 14% relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions.

[0185] In other aspects, the present invention is directed to a cell of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, as well as to a cell, seed, plant or plant part, or a protoplast of a cell, seed, plant, or plant part, produced therefrom. In some embodiments, the plant part is an ovule, a leaf, pollen, a seed, an embryo a root, a root tip, a pod, a flower, or a stalk. In other embodiments, the plant part is pollen or an ovule.

[0186] Another embodiment of the present invention is directed to a cell, seed, plant, or plant part having the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5 6, 7, 8 , 9, 10, 11, 12, or 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata*.

[0187] The present invention also includes a method of producing a *Brassica carinata* plant comprising a desired trait, as well as a cell, seed, plant, or plant part thereof, by introducing a nucleic acid construct conferring the desired trait into the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, using polyethylene glycol (PEG) mediated nucleic acid uptake, electroporation, ballistic infiltration using nucleic acid coated microprojectiles (gene gun), an *Agrobacterium* infiltration-based vector, or a plant virus-based vector. In some embodiments, the nucleic acid construct comprises a transgene. In other embodiments, the nucleic acid construct comprises an RNAi construct. In other embodiments, the *Brassica carinata* plant comprises the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata*. In other embodiments, the desired trait is selected from the group consisting of male sterility, disease resistance, fungal resistance, pest resistance, herbicide tolerance, abiotic stress tolerance, and altered metabolism. In other embodiments, the desired trait is herbicide tolerance and the herbicide is selected from, but not limited to, the group consisting of glyphosate, glufosinate, imidazolinones, and auxin analogues such as 2,4-D and dicamba.

[0188] In another aspect, the invention is directed to the use of a plant of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, to produce a *Brassica carinata* plant comprising a new trait, wherein the new trait is introduced by exposing seedlings or microspores to a mutagenic agent. In some embodiments, the mutagenic agent is ethyl methanesulfonate, N-ethyl-N-nitrosourea, or x-ray, gamma or ultraviolet radiation.

[0189] In another aspect, the invention is directed to a cell of a plant of a *Brassica carinata* plant produced from the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, wherein the *Brassica carinata* plant comprises a new trait and is produced by a method comprising exposing seedlings or microspores to a mutagenic agent and allowing the surviving fraction to develop into mature plants. In some embodiments, the mutagenic agent is ethyl methanesulfonate, N-ethyl-N-nitrosourea, or x-ray, gamma or ultraviolet radiation.

[0190] In another aspect, the invention is directed to a method of producing a commercial crop of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, or a progeny *Brassica carinata* plant produced therefrom by any of methods described herein. In another aspect, the invention is directed to a method of producing a commercial plant product from the commercial crop. In some embodiments, the commercial plant product comprises oil, meal, protein isolate, or biofumigant. In other embodiments, the commercial plant product comprises meal or protein isolate having a total glucosinolate content no greater than 30 micromoles per gram (dry weight).

[0191] In another aspect, the invention is directed to a method of producing crushed, non-viable seed of the *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, or a *Brassica carinata* plant produced by any of methods described herein.

[0192] In another aspect, the invention is directed to a method of biofumigation comprising, growing a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having reduced total glucosinolate content relative to the total glucosinolate content of seed from existing commercial or check plants of *Brassica carinata* grown in the same location under the same environmental conditions, described herein, or a *Brassica carinata* plant produced by any

of methods described herein in a field, collecting the *Brassica carinata* plant biomass between flowering and seed set, chopping the biomass, and incorporating the chopped biomass into the soil.

[0193] The citation of any publication herein is not an admission that the publication is prior art with respect to the present application.

[0194] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the scope of the appended claims.

[0195] It is to be understood that any numerical value inherently contains certain errors necessarily resulting from the standard deviation found in the respective testing measurements. Also, as used herein, the term “about” generally means within 10%, 5%, 1%, or 0.5% of a given value or range. Alternatively, the term “about” means within an acceptable standard error of the mean when considered by one of ordinary skill in the art. Unless indicated to the contrary, the numerical parameters set forth in the present disclosure and attached claims are approximations that can vary as desired. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0196] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

[0197] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, *i.e.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, *i.e.*, "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A

only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0198] As used herein in the specification and in the claims, "or" should be understood to encompass the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, *i.e.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items.

[0199] As used herein, whether in the specification or the appended claims, the transitional terms "comprising", "including", "carrying", "having", "containing", "involving", and the like are to be understood as being inclusive or open-ended (*i.e.*, to mean including but not limited to), and they do not exclude unrecited elements, materials or method steps. Only the transitional phrases "consisting of" and "consisting essentially of", respectively, are closed or semi-closed transitional phrases with respect to claims and exemplary embodiment paragraphs herein. The transitional phrase "consisting of" excludes any element, step, or ingredient which is not specifically recited. The transitional phrase "consisting essentially of" limits the scope to the specified elements, materials or steps and to those that do not materially affect the basic characteristic(s) of the invention disclosed and/or claimed herein.

EXAMPLES

[0200] Example 1: Field Trials

[0201] Small plot field experiments were conducted in Western Canada, in selected southeast or northern states of the United States, or in Uruguay, using best agronomic practices for carinata as describe the “Carinata Management Handbook” for the southeastern United States (https://agrisoma.com/ckfinder/userfiles/files/2017_18_SE_Handbook.pdf) or for the northern tier United States and Canadian prairies (http://growcarinata.com/ckfinder/userfiles/files/US%20Northern%20Plains_handbook_2018.pdf). Several newly developed carinata varieties, including DH-146.047, DH-146.194, DH-146.214, and DH-146.842 were grown in a Randomized Complete Block Design trial (RCBD), comprising 4 replicate plots (unless otherwise noted) along with plots of check varieties AAC-A120 and/or AGR044-312D-HP11 (henceforth referred to as “AGR044-HP11” or “HP11”). During the trials, the experimental lines and check lines were closely observed, and descriptions of phenotypic characteristics and agronomic traits were recorded. After plants had reached maturity, seed was collected for subsequent NIR based seed quality analysis (as described in the definitions section). Least square mean values from replicated data sets were calculated using the REML model and pairwise comparisons carried out using Tukey’s test at the 5% probability level. All quantitative data analysis was carried out using JMP 13 or JMP 14(SAS) .

[0202] Example 2: Characteristics of DH-146.047, DH-146.194, DH-146.214, and DH-146.842 grown in northern US and Canada.

[0203] The northern tier states of the US, such as North Dakota (ND) and its neighbours, as well as the adjacent southern Canadian prairie regions of Alberta and Saskatchewan (SK) constitute an important *Brassica* oilseed growing region. Evaluation of new carinata varieties in this region allows for identification of those varieties that are best adapted to growing in the local environment, which comprises a semiarid environment with warm summer and cold winter season. Under such conditions, carinata varieties which are productive under long daylight spring-summer cultivation in semiarid environments would prove advantageous to local farmers. Field trials are carried out annually at several sites in the region as described in Example 1 to

identify best candidates for commercialization based on agronomic traits, seed yield and quality as well as multi-year performance.

Trial Year	Variety	Trial sites
2017	DH-146.047	Devil's Lake ND, Milestone SK, Outlook SK
	DH-146.194	Carrington ND, Milestone SK, Outlook SK
	DH-146.214	Carrington ND, Devil's Lake ND, Milestone SK
	DH-146.842	Carrington ND, Indian Head SK, Milestone SK, Outlook SK
2018	DH-146.047	Brookings ND, Outlook SK
	DH-146.194	
	DH-146.214	
	DH-146.842	

[0204] The detailed phenotypic information provided in Tables 1 and 2 is based on data collected from small plot, RCBD field experiments conducted in northern US states and Canada, as described in Example 1. Each characteristic was assessed as described in DEFINITIONS section, above.

Table 1: Agronomic characteristics observed in 2017 field trials in northern US and Canada (measurements that are significantly different are indicated by *)

Trait	DH-146.047	AAC-A120	DH-146.197	AAC-A120	DH-146.214	AAC-A120	DH-146.842	AAC-A120
Early vigour (1-7 scale)	4.5	4.9	5.2	5.4	5.4	5.6	5.3	5.3
Days to first flower	ND	ND	53.4*	48.6*	50.6	50.7	51.2	48.6
Days to end flowering	ND	ND	74.5	77.3	72.0	75.8	ND	ND
Flowering duration (days)	ND	ND	24.3*	31.3*	26.0	28.8	ND	ND
Days to maturity	ND	ND	108.2	106.4	113.2	111.3	110.4	106.4
Bottom pod canopy (cm)	67.5	64.4	47.1	46.4	72.6	74.0	45.4	46.4
Canopy height (cm)	111.9	104.4	94.9	96.7	111.3	113.7	83.8	96.7
Depth of canopy (cm)	44	40	49.32	53.14	38.9	39.7	42.0	53.1
Lodging resistance (1-7)	5.8	5.5	6	5.1	4.5	4.1	5.9	5.1

Table 2: Agronomic characteristics observed in 2018 field trials in northern US and Canada

Trait	DH-146.047	DH-146.194	DH-146.214	DH-146.842	HP11	AAC-A120
Days to first flower	55	59.75	54.00	58.25	56	56
Days to end flowering	70	77.50	71.75	75.75	74	73
Flowering duration (days)	15	17.75	17.75	17.50	18	17
Bottom pod canopy (cm)	25.00	27.50	25.75	22.25	30.25	33.25
Canopy height (cm)	84.00	78.75	83.00	61.50	82.25	84.25
Depth of canopy (cm)	59.00	51.25	57.25	39.25	52.00	51.00

[0205] *Brassica carinata* DH-146.047, DH-146.194, DH-146.214, and DH-146.842 were observed to be similar to check AAC-A120 in most phenotypic characteristics, with the following exceptions:

Canopy In both the 2017 and 2018 trials, plants of DH-146.047 were slightly taller in stature than check variety; bottom of canopy was observed to be slightly higher, and canopy depth was observed to be slightly larger, than the check variety. In contract, plants of DH-148.842 were shorter in stature than check plants and canopy depth was observed to be smaller than the check plants in both the 2017 and 2018 trials.

[0206] Agronomic data accumulated from the trials described above indicate that *Brassica carinata* DH-146.047, DH-146.194, DH-146.214, and DH-146.842 possess traits consistent with those of an existing commercial line typically grown in the northern US and Canadian southern prairies and supports selection of these varieties as suitable candidates for commercial cultivation in this region.

[0207] Example 3: Grain yields from 2017 and 2018 field trials in northern US and Canada.

[0208] Table 3 (below) summarizes the results of yield analysis carried out on *Brassica carinata* DH-146.047, DH-146.194, DH-146.214, and DH-146.842 and check AAC-A120. Least square means (LSM) were calculated from replicate trials for each variety tested at each site using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses. Tukey's test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for each DH-146 and the check grown at the same location. Paired LSM values that share a common letter (letters columns) are not significantly different from one another.

Table 3: Grain yields from 2017 and 2018 field trials in northern US states and Canada

Year	Site	Variety	LSM Yield (kg/ha)	Letters
2017	Devil's Lake, ND	DH-146.047	4654	A
		AAC-A120	4055	AB
	Outlook, SK	DH-146.047	4462	A
		AAC-A120	4380	A
	Milestone, SK	DH-146.047	2140	ABC
		AAC-A120	2274	AB
	Carrington, ND	DH-146.194	2954	A
		AAC-A120	2086	BC
	Outlook SK	DH-146.194	3261	A
		AAC-A120	3434	A
	Milestone, SK	DH-146.194	1629	AB
		AAC-A120	1827	AB
	Carrington, ND	DH-146.214	2960	AB
		AAC-A120	2545	ABCDEF
	Devil's Lake, ND	DH-146.214	4506	ABC
		AAC-A120	4046	ABCD
2018	Brookings, ND	DH-146.214	2210	ABC
		AAC-A120	2000	BCDEF
		DH-146.842	2668	AB
		AAC-A120	2086	BC
		DH-146.842	2939	A
	Outlook, SK	AAC-A120	3057	A
		DH-146.842	1985	A
		AAC-A120	1827	AB
		DH-146.842	3309	A
		AAC-A120	3434	A
	Brookings, ND	DH-146.047	1443	AB
		DH-146.194	1603	AB
		DH-146.214	1837	AB
		DH-146.842	1745	AB
		AAC-A120	1849	A
	Outlook, SK	DH-146.047	3247	A
		DH-146.194	3055	A
		DH-146.214	ND	A
		DH-146.842	3111	A
		AAC-A120	3732	A

[0209] While the yield data reflect considerable variation between sites, at each individual site the difference in LSM yields between a given DH-146.xxx variety and the commercial check

AAC-A120 were small and only found to be significant for DH-146.194 grown at Carrington, ND in 2017.

[0210] The similar yield potentials for DH-146.047, DH-146.194, DH-146.214, and DH-146.842 and a commercial carinata line used as check, support selection of these varieties as suitable candidates for commercial cultivation in this region.

[0211] Example 4: Quality of carinata seed harvested from field trials conducted in northern US and Canada in 2017 and 2018.

[0212] Trials were conducted as described in Example 1. Seed quality data were obtained from NIR analysis as described above under DEFINITIONS, on replicated samples of harvested grain from small plot, RCBD field experiments. Least square means (LSM) were calculated for measurements of replicate samples using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses. Tukey's test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for DH-146.047 and the check variety (Table 4). Paired LSM values that share a common letter (letters columns) are not significantly different from one another.

Table 4: Seed quality of DH-146 varieties from 2017 and 2018 trials in northern US and Canada.

Quality	Year	Site	Variety	LSM	Letters
Seed Protein content, wt% of seed	2017	Combined sites	DH-146.047	26.06	DEFGHI
			AAC-A120	27.90	ABC
			DH-146.194	27.88	CD
			AAC-A120	29.35	BC
			DH-146.214	26.61	CDE
			AAC-A120	28.27	ABCD
			DH-146.842	27.47	D
			AAC-A120	29.35	BC
	2018	Brookings, ND	DH-146.047	27.20	CD
			DH-146.194	27.35	CD
			DH-146.214	27.48	BCD
			DH-146.842	26.35	D
			AAC-A120	29.50	AB
		Outlook, SK	DH-146.047	29.97	A
			DH-146.194	29.22	A
			DH-146.842	28.74	A
Seed Oil content, wt% of seed	2017	Combined sites	AAC-A120	31.62	A
			DH-146.047	46.26	BCDEFG
			AAC-A120	44.96	FGHI
			DH-146.194	45.64	A
			AAC-A120	43.12	BC

			DH-146.214 AAC-A120	46.59 44.28	ABC DEFG						
			DH-146.842 AAC-A120	45.75 43.12	A BC						
			2018	Brookings, ND	DH-146.047 DH-146.194 DH-146.214 DH-146.842 AAC-A120	45.46 47.28 46.92 49.05 43.88	CDE ABC BC A E				
					Outlook, SK	DH-146.047 DH-146.194 DH-146.842 AAC-A120	42.86 45.55 45.47 41.54	AB A A ABC			
						Glucosinolates (μmol/g)	2017	Combined sites	DH-146.047 AAC-A120	71.75 94.92	GHI ABC
									DH-146.194 AAC-A120	74.0 102.9	E B
									DH-146.214 AAC-A120	62.58 97.07	L CDEF
			DH-146.842 AAC-A120	73.6 102.9	E B						
			2018	Brookings, ND	DH-146.047 DH-146.194 DH-146.214 DH-146.842 AAC-A120		45.09 40.70 40.43 33.83 65.43	BCDE DE CDE E AB			
					Outlook, SK		DH-146.047 DH-146.194 DH-146.842 AAC-A120	79.25 75.55 70.96 102.15	C C C AB		
Erucic Acid, wt% of total fatty acids	2017	Combined sites	DH-146.047 AAC-A120				39.97 40.64	EFGHI DEFG			
			DH-146.194 AAC-A120				42.31 43.04	CD CD			
			DH-146.214 AAC-A120				43.51 41.81	B CDEFG			
			DH-146.842 AAC-A120	42.41 43.04	CD CD						
	2018	Brookings, ND	DH-146.047 DH-146.194 DH-146.214 DH-146.842 AAC-A120	43.19 42.25 45.28 43.35 42.75	ABCDE CDE ABC BCDE BCDE						
			Outlook, SK	DH-146.047 DH-146.194 DH-146.842 AAC-A120	46.25 47.57 45.28 45.81	CD BCD CD CD					
	Total Saturates, wt% of total fatty acids			2017	Combined sites	DH-146.047 AAC-A120	5.96 5.92	D DE			
						DH-146.194 AAC-A120	5.90 5.71	BCD EF			
						DH-146.214 AAC-A120	6.01 5.86	CDEF FGHIJ			
		DH-146.842 AAC-A120	5.97 5.71			BC EF					
2018		Brookings, ND	DH-146.047 DH-146.194	5.67 5.85	DE CDE						

			DH-146.214	6.04	ABCD
			DH-146.842	5.83	DE
			AAC-A120	5.90	BCDE
		Outlook, SK	DH-146.047	5.72	CD
			DH-146.194	5.68	CD
			DH-146.842	5.93	ABC
			AAC-A120	5.56	CD

Seed oil content Seed oil content was higher than that of the check variety for DH-146.194, DH-146.214 and DH-146.842 in grain harvested from 2017 trials, as well as from the 2018 trials in Brookings, ND. However, no differences in seed oil content relative to the check variety were observed in grain harvested from the 2018 trials in Outlook, SK.

Seed protein content Seed protein content was lower than that of the check variety for DH-146.047 and DH-146.842 in grain harvested from 2017 trials. Seed protein content was lower than that of the check variety for DH-146.047, DH-146.194, and DH-146.842 in grain harvested from 2018 trials in Brookings, ND. However, no differences in seed protein content relative to the check variety were observed in grain harvested from the 2018 trials in Outlook, SK.

Glucosinolates For all DH-146 varieties from all of the 2017 and 2018 trials, the seed glucosinolate content was reduced by 20 to 35 μmol GSL per g of seed, representing relative reductions in GSL content of 22 to 49% relative to the GSL content of seed from the check variety.

Erucic acid Oil from grain produced by the DH-146 varieties in 2017 and 2018 has statistically similar levels of erucic acid (C22:1) compared to oil from grain of check AAC-A120 grown at the same trial site.

Total saturates Oil from grain produced by the DH-146 varieties in 2018 has statistically similar proportion of saturate fatty acids compared to oil from grain of check AAC-A120 at both sites. In the 2017 trials, Oil from grain from DH-146.194 and DH-146.842 had

higher levels of saturated fatty acids than oil from grain of the check variety.

[0213] The fatty acid profile of seed oil from the four DH-146 varieties, as determined by NIR analysis, was also compared to that of seed from check AAC-A120 and the results, expressed as the percentage by weight (mean of 4 samples) for each of the major fatty acid constituents of the oil is shown in Table 5. Paired LSM values that share a common letter (letters columns) are not significantly different from one another.

Table 5 Fatty acid profile of oil from DH-146 varieties grown in Northern US and Canada in 2018

		C18:1		C18:2		C18:3		C20:1	
Location	Variety	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
Brookings, ND	DH-146.047	5.77	ABCD	15.03	CDE	14.47	BC	8.91	ABC
	DH-146.194	6.53	ABC	14.15	DE	16.08	A	8.08	CD
	DH-146.214	5.49	BCD	13.57	E	15.23	AB	7.96	CD
	DH-146.842	6.58	ABC	14.40	CDE	15.75	A	7.15	D
	AAC-A120	6.33	ABCD	15.40	BCD	14.08	CD	9.15	AB
Outlook, SK	DH-146.047	5.38	BC	14.74	BC	14.82	B	7.73	BC
	DH-146.194	4.36	BCD	13.71	CD	16.57	A	7.41	BC
	DH-146.842	6.42	B	14.23	BCD	15.65	AB	6.76	C
	AAC-A120	6.06	BC	14.93	BC	15.23	AB	8.51	ABC

		MONO		POLY		LCFA		VLCFA	
Location	Variety	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
Brookings, ND	DH-146.047	60.01	BCD	34.07	A	39.88	BCD	60.12	CDE
	DH-146.194	59.18	DE	34.40	A	46.15	A	53.85	F
	DH-146.214	60.65	B	32.86	B	43.06	AB	56.94	EF
	DH-146.842	60.30	BC	34.15	A	43.93	AB	56.08	EF
	AAC-A120	58.45	E	34.13	A	40.50	BC	59.50	DE
Outlook, SK	DH-146.047	59.61	BCD	33.73	AB	38.38	AB	61.62	CD
	DH-146.194	59.00	BCD	34.20	A	38.49	AB	61.51	CD
	DH-146.842	59.76	BCD	33.64	AB	41.74	A	58.26	D
	AAC-A120	57.51	D	34.30	A	37.81	AB	62.19	CD

[0214] The fatty acid profile of the oil from grain produced by the DH-146 varieties at Outlook, SK in 2018 is statistically similar to that of the oil from grain produced by the commercial check variety. The fatty acid profile of the oil from grain produced by the DH-146 varieties at Brookings, ND in 2018 differed from that of the oil from grain produced by the commercial check variety as follows:

C18:2 Oil from grain produced by DH-146.214 contained lower levels of linoleic acid compared to oil from grain produced

by the check variety.

C18:3	Oil from grain produced by DH-146.194, DH-146.214, and DH-146.842 contained higher levels of linolenic acid compared to oil from grain produced by the check variety.
C20:1	Oil from grain produced by DH-146.194, DH-146.214, and DH-146.842 contained lower levels of eicosenoic acid compared to oil from grain produced by the check variety.
MONO vs POLY unsaturated fatty acids	Oil from grain produced by DH-146.047, DH-146.214, and DH-146.842 contained higher levels of mono-unsaturated fatty acids compared to oil from grain produced by the check variety. However, only the oil from grain produced by DH-146.214 showed a corresponding reduction in the levels of poly-unsaturated fatty acids compared to oil from grain produced by the check variety.
LCFA vs VLCFA	Oil from grain produced by DH-146.194 contained higher levels of long-chain fatty acids, and lower levels of very long chain fatty acids, compared to oil from grain produced by the check variety.

[0215] Seed from the DH-146 varieties have similar or slightly higher oil content compared to seed from the check variety. Based on yield equivalence between DH-146 varieties and the check line (shown in Example 3), this could result in similar or even higher amounts of oil recovered per hectare for fields planted with DH-146 varieties.

[0216] In these trials, the DH-146 varieties produced seed with a highly significant 20-35 $\mu\text{mol/g}$ lower total GSL content than the commercial check line. These significantly lower levels of GSL found in seed of the DH-146 varieties could positively impact the quality of oil and meal feedstocks produced from its grain. Even lower GSL inclusion rates in carinata meal may be achieved through application of downstream processing technologies, such as that described in U.S. Publication No. US2018/0042266 A1.

[0217] Seed of some of the DH-146 varieties produced in these trials contained a slightly lower protein content than that of the check line. However, the difference was small (~2-3 wt%) and meal produced from processing of this seed would still be expected to contain > 40% protein content (on a dry weight basis), representing an advantage with respect to competing *Brassica* oil seed meals such as canola meal.

[0218] Based on the potential for oil yields equivalent to or slightly higher than oil yields from the existing commercial variety, as well as the seed quality attributes discussed above, varieties DH-146.047, DH-146.194, DH-146.214, and DH-146.842 are suitable candidates for commercial exploitation in the northern US states and Canada, and possibly other geographical regions.

[0219] Example 5: Characteristics of DH-146.047, DH-146.194, DH-146.214, and DH-146.842 when grown in Southeastern United States

[0220] The southeastern states of Florida, Georgia, Alabama and others constitute an important carinata growing region. Farmers in this region would benefit from a crop that can be grown during the winter months as a cover crop, replacing the normal winter fallow in their rotations, allowing them an additional cash crop option while providing soil benefits that may in turn benefit other crops in their rotation. To identify varieties with suitable productivity and agronomic properties in the low daylight winter cropping scenario, field trial evaluations of new carinata varieties in this region are carried out annually at several sites in the region to identify best candidates for commercialization based on agronomic traits, seed yield and quality, as well as multi-year performance.

[0221] As described in Example 1, replicated field trials afford the opportunity to compare experimental varieties in terms of their morphological phenotypes and agronomic traits. In 2016-2017, field trials were carried out in multiple sites in Central and North Florida and one of the trial sites (Quincy, FL) provided extensive phenotypic observations, summarized in Table 6. Phenotypic observations from 2017-18 field trials carried out in multiple sites in Central and North Florida, Georgia, and Alabama are summarized in Table 7. Each characteristic was assessed as described in DEFINITIONS section, above, with the following exceptions and additions:

- Height: from soil level to topmost visible node. Recorded at physiological maturity.
- *Sclerotinia* incidence: expressed as a % of symptomatic plants in a 39” × 48” area (data collected 2-4 days before harvest)

[0222] Least square means (LSM) were calculated for measurements of replicate samples using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses. Tukey’s test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for DH-146.842 and the check variety (Tables 6 and 7). Paired LSM values that share a common letter (letters columns) are not significantly different from one another.

Table 6: Morphological traits of DH-146.842 grown in southeast US in 2016-17

Trait	Variety	DH-146.842	Letters
Days to first bolt	DH-146.842	88.0	B
	AGR044-HP11	84.3	BCD
Days to 50% bolt	DH-146.842	93.5	B
	AGR044-HP11	89.5	CDE
Days to first flower	DH-146.842	96	B
	AGR044-HP11	93.5	B
Days to 50% flower	DH-146.842	105.0	B
	AGR044-HP11	105.5	B
Days to last flower	DH-146.842	132.0	ABC
	AGR044-HP11	135.5	A
Depth of canopy (cm)	DH-146.842	76.20	A
	AGR044-HP11	79.25	A
Stand (plants/m ²)	DH-146.842	37.5	A
	AGR044-HP11	42.4	A
Plant height (cm)	DH-146.842	169	ABCD
	AGR044-HP11	186	A
Pod shatter loss (kg/ha)	DH-146.842	161.0	ABCD
	AGR044-HP11	189.5	ABCD
<i>Sclerotinia</i> incidence, %	DH-146.842	6.3	CD
	AGR044-HP11	8.3	CD
TKW (weight (g) of 1000 seeds)	DH-146.842	4.2	DEF
	AGR044-HP11	4.8	BCDE

Table 7: Morphological traits of DH-146.842 grown in southeast US in 2017-18

		Stand (plants per m ²)		Freeze surv/rec @104 days (%)*		Days to 50% bolt		Days to 50% flower		Plant height (cm)	
Location	Variety	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
Midville, GA	842	77.0	A	78.8	AB	112	AB	ND		76.5	CDEF
	HP11	77.8	A	67.5	AB	110	ABC	ND		81.0	BCDEF

Tifton, GA	842	73.0	AB	ND	98.1	A	105	ABC	ND
	HP11	74.6	AB	ND	93.0	ABC	103	ABCDEF	ND
Shorter, AL	842	58.4	AB	ND	ND		136	ABC	ND
	HP11	63.6	AB	ND	ND		137	AB	ND
Quincy, FL (late)	842	64.9	DEF	ND	93.4	AB	100	CDE	ND
	HP11	90.6	ABC	ND	94.5	AB	104	AB	ND
Live Oak, FL	842	65.4	ABC	98.8	94.3	AB	100	BCD	107
	HP11	65.8	ABC	100	89.4	DEF	97.6	CDEF	110
Jay, FL	842	57.3	BCD	45.0	93.9	A	102	A	102
	HP11	56.8	BCD	54.4	91.6	BCD	102	A	121

ND = not determined

*freeze survival in Midville, GA; freeze recovery in Live Oak and Jay, FL.

[0223] During the field trials conducted in southeastern US states in the winters of 2016-17 and 2017-18, *Brassica carinata* DH-146.842 was observed to be similar to check AAC-A120 in most phenotypic characteristics, with the following exceptions:

Plant height: Plants of DH-146.842 were found slightly shorter in stature than check AGR044- HP11, but differences were not statistically significant.

Bolting Plants of DH-146.842 bolted later than plants of the check variety in Quincy FL in 2017 and at most sites in 2018.

[0224] In summary, DH-146.842 exhibits agronomic traits consistent with those of an existing commercial line grown in the SE US region, making it a suitable candidate for commercial cultivation in the SE USA.

[0225] Example 6: Grain yields of DH-146.842 grown in winter field trials in southeast US states

[0226] To assess the yield potential of new and experimental carinata varieties, including DH-146.842, relative to commercial check lines, a series replicated yield trials designed as described in Example 1 were carried out during winters of 2016-2017 and 2017-18 in southeast US. In 2016-17, trials were carried out at four sites; however, all but one of the sites (Quincy, FL) demonstrated coefficients of variation (C of V) for yields too high (>15%) to be considered reliable; therefore, only yield data from Quincy were considered. In 2017-18, trials were carried out at multiple sites in Florida, Georgia, and Alabama.

[0227] After maturity had been reached, seeds were harvested from the center 5' of each plot (5 rows), cleaned and dried in a forced air oven at 50°C for at least 72h, subsequently weighed for yield determination and the seed analyzed for test weight and moisture using a Steinlite SL95 Moisture Meter. Reported yields, tabulated in Table 8, have been adjusted to 8% moisture.

[0228] Least square means (LSM) were calculated from replicate trials for each variety tested at each site using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses.

Tukey's test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for each DH-146 variety and the check variety grown at the same location. Paired LSM values that share a common letter (letters columns) are not significantly different from one another.

Table 8: Grain yields of DH-146.842 grown in southeast US in winter 2016-17 and 2017-18

	Location	Variety	LSM Yield	Letters
2016-17	Quincy, FL	DH-146.842	2656	AB
		AGR044-HP11	2106	A
2017-18	Midville, GA	DH-146.842	1674	A
		AGR044-HP11	1661	A
	Tifton, GA	DH-146.842	2482	ABC
		AGR044-HP11	2208	C
	Shorter, AL	DH-146.842	3195	ABCD
		AGR044-HP11	3140	ABCD
	Quincy, FL (late)	DH-146.842	3611	A
		AGR044-HP11	3178	AB
	Quincy, FL (early)	DH-146.842	2255	A
		AGR044-HP11	3114	A
	Live Oak, FL	DH-146.842	2584	ABC
		AGR044-HP11	2399	BCDE
	Jay, FL	DH-146.842	2900	AB
		AGR044-HP11	2223	AB

[0229] While the yield data reflect considerable variation between sites, at each individual site the difference in LSM yields between DH-146.842 and the commercial check AAC-A120 was not found to be significant. The similar yield potentials for DH-146.842 and a commercial carinata variety used as check line support selection of DH-146.842 as a suitable candidate for commercial cultivation in this region.

[0230] Example 7: Quality of carinata seed harvested from winter field trials in southeast US

[0231] Trials were conducted in southeastern US states as described in Example 1. Seed quality data were obtained from NIR analysis, as described above under DEFINITIONS, on replicated samples of harvested grain from small plot, RCBD field experiments. Least square means (LSM) were calculated for measurements of replicate samples using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses. Tukey's test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for DH-146.842 ("842") and the check variety (AGR044-312D-HP11, or "HP11"). Results are summarized in Table 9. Paired LSM values that are statistically different from one another are indicated by an asterisk (*); otherwise, the values are not considered to be statistically different from one another.

Table 9: Seed quality characteristics of DH-146.842 (842) grown in southeast US

Year	Location	Line	Oil, wt % of seed		Glucosinolates, μmol/ g seed		Erucic Acid, wt % of seed		Protein, wt % of seed		Total Saturates, wt % of seed	
			LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
2016 -17	Combined sites	842	49.0	AB	64.7	D	41.9	CDE	24.3	F	5.8	ABC
		HP11	45.8	C	83.6	C	44.2	B	25.1	EF	5.5	CD
2017 -18	Midville GA	842	46.4	A	73.3	A	38.3	A	23.6	A	ND	
		HP11	46.4	A	74.5	A	38.4	A	23.6	A		
	Tifton GA	842	51.9	AB	58.7	HI	39.6	EFG	22.8	BCD	6.1	BCD
		HP11	50.7	ABC	68.8	FGH	42.5	BC	21.7	D	5.9	DE
	Shorter AL	842	48.6	ABC	64.7	F	40.8	EFG	25.3	BCD	6.0	ABCD
		HP11	47.0	ABCDE	79.0	CDE	42.8	CD	24.4	D	5.9	CD
	Quincy FL (late)	842	48.2	AB	72.9	E	42.4	FG	25.9	DE	6.1	BCDE
		HP11	47.9	ABCD	93.3	D	46.1	BCD	25.5	DE	5.7	FG
	Quincy FL (early)	842	55.8	A	35.5	F	42.0	FG	18.9	D	6.0	BCDE
		HP11	51.4	CDE	58.2	CDE	45.6	AB	20.6	ABCD	5.8	FG
	Live Oak FL	842	46.3	A	65.2	H	38.4	EFG	27.0	E	5.8	CDE
		HP11	43.6	BCDE	79.9	EFG	41.7	ABC	27.1	E	5.8	CDE
	Citra FL	842	46.6	A	50.6	J	41.4	C	26.5	BCD	6.0	BCD
		HP11	43.8	ABCDE	72.6	FG	45.1	A	27.7	ABCD	5.8	DEFG
	Jay FL	842	53.7	AB	47.8	F	42.9	EF	20.4	C	6.1	ABCDE
		HP11	51.8	BCDE	60.4	CDE	45.2	ABC	19.9	C	6.0	ABCDE

[0232] The results of the seed quality analysis of DH-146.842 grown in southeast US during the winter of 2016-2017 and 2017-18 are summarized below can be summarized as follows:

Seed oil content Depending on the year and location, seed from DH-146.842 has statistically similar or higher oil content than seed from check

AGR044- HP11.

Seed protein content Seed produced from DH-146.842 in all trials has a similar protein content to that of check AGR044-HP11.

Glucosinolates In all but one of the 2017 and 2018 trials, the seed glucosinolate content for DH-146.847 was reduced by 10 to 23 μmol GSL per g of seed, representing relative reductions in GSL content of 14 to 39% relative to the GSL content of seed from the check variety. Such reduction may positively impact both meal and oil value. Reduction of grain GSL levels can result in lower sulphur contamination of oil extracted during grain crush operations and lower meal GSL allows for higher inclusion rates of meal in animal feed products.

Erucic acid In all but one of the 2017 and 2018 trials, oil from DH-146.842 grain contains significantly lower levels of erucic acid (C22:1) compared to oil from grain of check AGR044- HP11.

Total saturates At most trial sites in 2017 and 2018, oil from DH-146.842 grain shows similar levels of total saturates as oil from grain of check AGR044- HP11. However, oil from grain harvested from the two Quincy FL trials in 2018 contained a higher proportion of saturated fatty acids than oil from grain of the check variety.

[0233] The fatty acid profile of seed oil from DH-146.842 (“842”), as determined by NIR analysis, was also compared to that of the AGR044-312D-HP11 (“HP11”) check line and the results, expressed as the percentage by weight (mean of 4 samples) for each of the major fatty acid constituents of the oil are shown in Table 10.

Table 10: Fatty acid profile of oil from DH-146.842 grown in southeast US in 2016-17 (Florida, combined sites and 2017-18

Year	Location	Variety	C18:1		C18:2		C18:3		C20:1	
			LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
2016-17	FL, combined sites	842	8.80	B	14.28	D	15.31	AB	6.94	EF
		HP11	7.87	BC	14.47	CD	14.27	CD	8.35	D
2017-	Midville,	842	9.53	A	16.13	A	12.43	A	10.38	A

18	GA	HP11	9.50	A	16.15	A	12.65	A	10.25	A
	Tifton, GA	842	10.98	AB	14.26	DE	13.45	BC	9.30	EFG
		HP11	9.30	DEF	14.45	D	12.28	DEF	10.23	BCD
	Shorter, AL	842	9.83	ABC	14.69	DEF	13.30	B	8.84	FG
		HP11	8.48	DE	14.91	DEF	11.94	DE	10.18	CD
	Quincy, FL (late)	842	7.83	AB	14.28	ABC	13.00	B	8.98	CDE
		HP11	5.13	CD	14.51	ABC	11.96	CD	9.37	CDE
	Quincy, FL (early)	842	10.29	BCD	12.29	FGH	15.00	B	9.07	GH
		HP11	7.39	GH	12.12	FGH	13.69	D	10.37	DE
	Live Oak, FL	842	9.72	BCD	15.09	DEFG	15.55	A	8.64	H
		HP11	7.98	EFG	15.80	BCDEF	13.71	EFG	9.85	ABCDE
	Citra, FL	842	8.10	CD	14.35	D	15.74	A	7.99	GH
		HP11	5.97	E	14.42	D	14.31	BCD	9.24	CDE
	Jay, FL	842	9.73	BCD	12.88	BCDE	14.41	B	8.51	G
		HP11	8.75	CDE	12.83	BCDE	12.72	DE	10.35	CD

			MONO		POLY		LCFA		VLCFA	
Year	Location	Variety	LSM	Letters	LSM	Letters	LSM	Letters	LSM	Letters
2016-17	FL,combined sties	842	60.44	ABCD	33.72	BC	47.31	A	52.69	D
		HP11	60.59	ABCD	33.26	BCD	42.22	CD	57.78	AB
2017-18	Midville, GA	842	61.93	A	31.40	A	39.40	A	60.60	A
		HP11	61.15	A	32.13	A	41.23	A	58.78	A
	Tifton, GA	842	63.38	BCD	30.81	BC	41.94	AB	58.06	FG
		HP11	63.95	ABC	29.94	CDEF	35.54	DEF	64.46	BCD
	Shorter, AL	842	62.25	DE	31.44	BCD	40.59	AB	59.41	IJ
		HP11	62.65	CD	30.58	DEFG	34.69	DEFG	65.31	DEFG
	Quincy, FL (late)	842	63.05	BCD	30.66	DE	39.05	ABC	60.95	DEF
		HP11	63.72	BC	29.94	CDE	32.28	DE	67.72	BC
	Quincy, FL (early)	842	63.28	CDE	31.31	B	41.07	B	58.93	H
		HP11	64.00	BC	30.09	DEF	33.40	EFGH	66.60	BCDE
	Live Oak, FL	842	58.92	DEF	34.73	ABC	46.33	A	53.67	G
		HP11	60.06	CD	33.42	DE	39.18	EF	60.82	BC
	Citra, FL	842	59.33	DEF	34.48	AB	45.85	A	54.15	G
		HP11	60.43	BCD	33.12	BCD	38.12	EFG	61.88	ABC
	Jay, FL	842	62.78	DEFGH	31.36	ABC	38.38	AB	61.62	EF
		HP11	64.23	BC	29.41	D	30.40	EF	69.60	AB

[0234] Depending on the location of the trial, the fatty acid profile of the oil from grain produced by DH-146.842 may show some differences relative to that of the oil from grain produced by the commercial check variety. The observed differences in the fatty acid profiles between oil from the grain of DH-146.842 and oil from the grain of the check variety are summarized below:

C18:1 For grain harvested from most sites, the DH-146.842 oil had higher levels of oleic acid than oil from the check variety.

C18:3 For grain harvested from most sites, oil produced by DH-146.842 contained higher levels of linolenic acid compared to oil from grain produced by the check variety.

C20:1	For grain harvested from most sites, oil from grain produced by DH-146.842 contained lower levels of eicosenoic acid compared to oil from grain produced by the check variety.
MONO vs POLY unsaturated fatty acids	Oil from grain produced by DH-146.842 at Jay FL contained lower levels of mono-unsaturated fatty acid and higher levels of poly-unsaturated fatty acids compared to oil from grain produced by the check variety. The oil from grain produced by DH-146.214 at Quincy FL (early) also contains lower levels of poly-unsaturated fatty acids compared to oil from grain produced by the check variety.
LCFA vs VLCFA	For grain harvested from most sites, oil from grain produced by DH-146.842 contained higher levels of long chain fatty acids, and lower levels of very long chain fatty acids, compared to oil from grain of the check variety.

[0235] As can be seen in Table 9, seed from DH-146.842 has similar or significantly higher oil content in relation to that of the commercial check variety. Based on yield equivalence between DH-146.842 and the check variety (shown in Example 4), this could result in higher amounts of oil recovered per hectare for fields planted with DH-146.842, an important commercial advantage. Based on the comparative fatty acid profile analysis shown in Table 10, depending on the trial conditions, DH-146.842 produces an oil with substantially similar composition and properties to that of the commercial check variety. Although the oil in DH-146.842 grain harvested from most of the trials in this region had a slightly lower erucic acid content than that of the check variety, at 38-42 wt% of the oil, it was still within the typical range seen for current commercial carinata varieties.

[0236] In these trials, DH-146.842 produced seed with a highly significant 10-23 µmol/g lower total GSL content than the commercial check line. The significantly lower levels of GSL found in the DH-146.842 seed could positively impact the quality of oil and meal feedstocks produced from its grain. Even lower GSL inclusion rates in carinata meal may be achieved through application of downstream processing technologies, such as that described in U.S. Publication No. US2018/0042266 A1.

[0237] DH-146.842 seed produced in these trials contained a slightly lower protein content than that of the check line. However, the difference was small (less than one percentage point) and meal produced from processing of this seed would still be expected to contain > 40% protein content, representing an advantage with respect to competing *Brassica* oil seed meals such as canola meal. Based on the potential for high oil yields relative to the existing commercial varieties as well as the seed quality attributes discussed above, DH-146.842 may be a suitable candidate for commercial exploitation in the southeast region of the US.

[0238] Example 8: Characteristics of DH-146.047, DH-146.194, DH-146.214, and DH-146.842 from winter 2017 and 2018 field trials in Uruguay.

[0239] Uruguay is an important commercial producer of *Brassica carinata*. Its climate lends itself to production of carinata as a short-day length winter cover crop often in rotation with summer grown crops such as soybean. To assess new carinata varieties for their suitability in commercial production in this region, small plot yield trials were carried out in Uruguay, primarily at two sites, La Estanzuela (LE) and Young (YO) in winters of 2017 and 2018, essentially as described in Example 1.

[0240] The detailed phenotypic information provided in Table 11 is based on data collected from small plot, RCBD field experiments conducted at two sites in Uruguay in the winter of 2017 and 2018, as described in Example 1. Each characteristic was assessed as described in DEFINITIONS section for all experiment carinata varieties entered in the trial, including DH-146.842, as well as the commercial check AGR044-HP11. The values given for days to first flower, days to 50% flower, days to last flower, and TKW (thousand kernel weight or thousand seed weight) are the mean of replicated measurements taken at the two sites, as indicated by “(av)” next to the values. The values given for first silique height, plant height, and lodging are the mean of replicated measurements at each site. The values for pod shattering loss (kg/ha) and stand (plants/m²) were measured only at La Estanzuela (LE).

[0241] Each characteristic was assessed as described in DEFINITIONS section. Table 11 provides additional data for morphological and agronomic characteristics for DH-146.842 and check AAC-A120.

Table 11: Characteristics of DH-146.047, DH-146.194, DH-146.214, and DH-146.842 from winter 2017 and 2018 field trials in Uruguay

Year	2017				2018			
Site	LE site		YO site		LE site		YO site	
	DH- 146.842	AGR044- HP11	DH- 146.842	AGR044- HP11	DH- 146.842	AGR044- HP11	DH- 146.842	AGR044- HP11
Days to 10% flower	82	87	80	82	117	119	83	82
Days to 50% flower	110	106	86	88	125	126	86	84
Days to last flower	132	132	107	106	151	153	107	103
Stand (plants/m2)	119	115	ND*	ND*	91	85	ND*	ND*
First silique height (m)	1.20	1.10	0.75	0.58	0.74	0.81	0.82	0.85
Plant height (m)	1.60	1.66	1.65	1.51	1.86	2.23	1.21	1.35
Pod shatter loss at harvest (kg/ha)	267	335	ND*	ND*	215	200	ND*	ND*
Lodging (0-5)	3.5	4.0	4.50	4.5	1	3		

*ND= not determined

[0242] Example 9: Yield of carinata seed from DH-146.842 from winter 2017 and 2018 field trials in Uruguay.

[0243] Table 12 (below) summarizes the results of yield analysis carried out on several new and experimental carinata varieties, including DH-146.842, and alongside check AGR044-HP11. Least square means (LSM) yields (in kg/Ha) were calculated from replicate trials for each variety tested at each site using a restricted maximal likelihood (REML) estimate, which can better describe data sets where some replicate data might be missing due to weather or disease related losses. Tukey's test was then applied pairwise to determine whether there were statistically significant differences between the LSM yields for DH-146.842 and the check variety. Paired LSM values that are statistically different from one another are indicated by an asterisk (*); otherwise, the values are not considered to be statistically different from one another.

Table 12: Yields of DH-146.842 grown in 2017 field trials in UY

Site	Variety	2017		2018	
		LSM Yield (kg/ha)	Letters	LSM Yield (kg/ha)	Letters
La Estanzuela	DH148.842	3188	A	4558	AB
	AGR044-HP11	4730	A	4922	AB
Yonge	DH148.842	3294	A	ND	
	AGR044-HP11	3800	A	ND	

[0244] While the yield data show some variation between sites, at each individual site the difference in LSM yields between DH-146.842 and the commercial check AAC-A120 was not found to be significant. The similar yield potentials for DH-146.842 and a commercial carinata variety used as check line support selection of DH-146.842 as a suitable candidate for commercial cultivation in this region.

[0245] Example 10: Quality of carinata seed harvested from winter field trials in Uruguay.

[0246] Small plot yield trials were carried out in Uruguay, primarily at two sites, La Estanzuela (LE) and Young (YO) in winters of 2017 and 2018, essentially as described in Example 1. Seed quality data were obtained from NIR analysis of replicated samples of harvested grain, as described under DEFINITIONS, above. Table 13 tabulates the main characteristics of DH-146.842 seed harvested from these trials in relation to those of the AGR044-HP11 check.

Table 13: Seed quality characteristics of DH-146.842 from winter field trials in Uruguay

Quality	Variety	2017	2018	
		Average of two sites	LSM	Letters
Protein, wt% of seed	DH-146.842	26.2	26.87	ABCDE
	AGR044-HP11	24.6	26.58	ABCDE
Oil, wt% of seed	DH-146.842	48.9	48.36	A
	AGR044-HP11	48.1	45.9	BCD
Glucosinolates ($\mu\text{mol/g}$)	DH-146.842	48.7	40.54	ABC
	AGR044-HP11	56.6	55.55	AB
Erucic Acid, wt% of total fatty acids	DH-146.842	45.6	42.32	CDE
	AGR044-HP11	46.8	45.66	AB
Total Saturates, wt% of fatty acids	DH-146.842	5.8	6.39	BCD
	AGR044-HP11	5.9	6.25	DEF
C18:1	DH-146.842	5.7	8.87	CD
	AGR044-HP11	5.6	6.79	DE
C18:2	DH-146.842	12.8	14.83	CDEFGH
	AGR044-HP11	12.8	14.25	GH
C18:3	DH-146.842	15.9	15.77	ABC
	AGR044-HP11	13.7	14.03	DEFG
C20:1	DH-146.842	7.2	6.50	EF
	AGR044-HP11	9.5	8.02	ABCD
Mono Unsaturated	DH-146.842	59.6	58.32	ABC
	AGR044-HP11	61.3	59.63	AB
Poly Unsaturated	DH-146.842	34.1	35.75	BCD
	AGR044-HP11	31.7	33.76	F
Long-chain FA ($\leq\text{C18}$)	DH-146.842	44.5	51.14	B
	AGR044-HP11	34.8	42.87	FGH
Very long-chain FA ($\geq\text{C20}$)	DH-146.842	55.5	48.86	G
	AGR044-HP11	65.2	57.13	ABC

[0247] The major seed quality trait characteristics of DH-146.842 grown in Uruguay in 2017 and 2018 are summarized below:

Seed oil content	Seed from DH-146.842 has similar or slightly higher oil content compared to seed from check AGR044-HP11.
Seed protein content	Seed from DH-146.842 has a similar or slightly higher protein content compared to seed from check AGR044-HP11.
Glucosinolates	The GSL content ($\mu\text{mol/g}$) of seed produced by DH-146.842 grown in Uruguay is 8 -15 μmol GSL per g seed lower than the GSL content in seed of the check AGR044-HP11. This represents a reduction of about 14-27% relative to the GSL content of seed produced by the check variety. Such reduction may positively impact both meal and oil value. Reduction of grain GSL levels can result in lower sulphur contamination of oil extracted during grain operations and lower meal GSL allows for higher inclusion rates of meal in animal feed products.
Erucic acid	Oil from DH-146.842 grain produced in 2017 had similar levels of erucic acid (C22:1) as oil from grain of the check variety. Oil from DH-146.842 grain produced in 2018 had lower levels of erucic acid (C22:1) as oil from grain of the check variety.
Fatty acids	Oil from DH-146.842 grown in Uruguay show slightly higher levels of LCFAs and slightly lower levels of VLCFAs.
Total saturates	Oil from DH-146.842 grain shows similar levels of total saturates as oil from grain of check AGR044- HP11.

[0248] As can be seen in Table 13, the DH-146.842 showed a similar oil or slightly higher content compared to that of the commercial check variety. Based on yield equivalence between DH-146.842 and the check line (shown in Example 9), this will result in similar amounts of oil recovered per hectare for fields planted with DH-146.842 and current commercial varieties.

[0249] In these trials, DH-146.842 produced seed with a significant 8 - 14 $\mu\text{mol/g}$ lower total GSL content than the commercial check variety. The significantly lower levels of GSL found in the DH-146.842 seed could positively impact the quality of oil and meal feedstocks produced from its grain. Even lower GSL inclusion rates in carinata meal may be achieved through application of downstream processing technologies, such as that described in U.S. Publication No. US2018/0042266 A1.

[0250] DH-146.842 seed produced in this trial contained a slightly higher protein content than that of the check variety; meal produced from processing of this seed would be expected to contain > 40% protein content, representing an advantage with respect to competing *Brassica* oil seed meals such as canola meal.

[0251] Based on the potential for similar oil yields as existing commercial varieties as well as the seed quality attributes discussed above, DH-146.842 may be a suitable candidate for commercial exploitation in Uruguay.

[0252] In summary, for all geographies tested to date, GSL level in seed produced by varieties DH-146.047, DH-146.194, DH-146.214, and DH-146.842 are consistently and, in the majority of trials, significantly lower, with reductions ranging from 14% to 48% relative to the GSL content of commercial check lines grown in the same geography (Examples 4, 7 and 10). Such reduction may positively impact both meal and oil value. Reduction of grain GSL levels can result in lower sulphur contamination of oil extracted during grain operations and lower meal GSL allows for higher inclusion rates of meal in animal feed products.

DEPOSIT

Applicant(s) have made a deposit of at least 2500 seeds of *Brassica carinata* varieties DH-146.047, DH-146.194, DH-146.214, and DH-146.842 with the NCIMB Ltd., Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, UK, AB21 9YA, under their Accession numbers 43005, 43006, 43007, and 43008, respectively. The seeds deposited with NCIMB on April 3, 2018 for each variety were taken from the seed stock maintained by Agrisoma Biosciences Inc., since prior to the filing date of this application. The deposit of seed of *Brassica carinata* varieties DH-146.047, DH-146.194, DH-146.214, and DH-146.842 will be maintained in the NCIMB depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent, whichever is longer, and will be replaced if it becomes nonviable during that period. Additionally, Applicant has satisfied all the requirements 37 C.F.R. §1.801 – 1.809, including providing an indication of the viability of the sample upon deposit. Applicant imposes no restrictions on the availability of the deposited material from NCIMB; however, Applicant has no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicant(s) do not waive any infringement of their rights granted under this patent or rights applicable to *Brassica carinata* varieties DH-146.047, DH-146.194, DH-146.214, and DH-146.842 under the Plant Variety Protection Act (7 USC 2321 et seq.).

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CLAIMS

1. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
2. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 20% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
3. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 22% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
4. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 8 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
5. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 10 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
6. A cell of a *Brassica carinata* plant carrying a genetic determinant that leads to production of seed having a total glucosinolate content that is reduced by at least 20 $\mu\text{mol/g}$ relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
7. The cell of any one of claims 1 through 6, wherein the source from which the genetic determinant is acquired is formed by plants grown from seed of which a representative sample is deposited with the NCIMB under deposit number 43005, 43006, 43007, or 43008, or from any cell, seed, plant part, or progeny thereof.

8. A cell of a *Brassica carinata* plant comprising a desired trait produced by a method comprising the steps of:
 - (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of claims 1 through 7;
 - (c) backcrossing the selected progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of claims 1 through 7 with plants produced from cell of a *Brassica carinata* plant of any one of claims 1 through 7 to produce backcross progeny plants; and
 - (d) growing seed from the resultant backcross progeny plants and selecting backcross progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of claims 1 through 7.
9. The cell of claim 8, wherein the method to produce the cell of a *Brassica carinata* plant comprising a desired trait further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
10. The cell of claim 8, wherein the method to produce the cell of a *Brassica carinata* plant further comprises repeating steps (c) and (d) until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
11. A cell of a *Brassica carinata* plant comprising a desired trait produced by a method comprising the steps of:

- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 with another *Brassica carinata* plant comprising the desired trait;
 - (b) growing the resultant F1 hybrid seed and selecting one or more F1 progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of claims 1 through 7;
 - (c) self-pollinating the F1 progeny plants that have the desired trait to produce further progeny plants;
 - (d) growing the resultant further progeny plants and selecting further progeny plants that have the desired trait and at least a portion of the genetic make up of the cell of a *Brassica carinata* plant of any one of claims 1 through 7
12. The cell of claim 11, wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 14% relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
13. The cell of claim 11 wherein steps (c) and (d) are repeated until the *Brassica carinata* plant has the desired trait and produces seed having a total glucosinolate content that is reduced by at least 8 μmol per g of seed relative to the total glucosinolate content of seed produced by a commercial or check *Brassica carinata* plant grown in the same location under the same environmental conditions.
14. A cell of a *Brassica carinata* plant comprising a desired trait produced by a method comprising:
- (a) crossing a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 13 with a plant of another *Brassicaceae* species comprising the desired trait;
 - (b) producing F1 plants using embryo rescue techniques to recover viable F1 plants from the cross or growing F1 seeds;
 - (c) self-pollinating the F1 plants that have the desired trait and carinata character;

- (d) producing F2 plants using embryo rescue techniques to recover viable F1 plants from the cross or growing F2 seeds;
 - (e) self-pollinating the F2 plants that have the desired trait and carinata character;
 - (f) producing progeny plants using embryo rescue techniques to recover viable F3 plants or growing F3 seeds;
 - (g) self-pollinating the progeny plants that have the desired trait and carinata character to produce further progeny plants; and
 - (h) selecting the progeny plants with the desired trait and carinata character.
15. The cell of claim 14, wherein steps (g) and (h) are repeated until the *Brassica carinata* plant has the desired trait and the physiological and/or morphological characteristics set forth in one or more of Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13, as determined at the 5% significance level when grown in the same location under the same environmental conditions as a plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7.
 16. A seed, plant, or plant part produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 15.
 17. A tissue culture of protoplasts or regenerable cells of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 15.
 18. A method of producing a commercial crop, the method comprising growing a plurality of plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 15.
 19. A method of producing a commercial plant product, the method comprising growing a plurality of plants produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 15 to produce a commercial crop and producing the commercial plant product from the commercial crop.
 20. A commercial plant product produced by the method of claim 19.

21. The commercial plant product produced by the method of claim 19 comprising oil, meal, protein isolate, or biofumigant.
22. The commercial plant product of claim 19 comprising meal or protein isolate, wherein the meal or protein isolate has a total glucosinolate content of no more than 30 micromoles per gram of meal (dry weight).
23. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 to produce seed, wherein the seed is produced by self-crossing or open pollination.
24. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 to produce F1 hybrid *Brassica carinata* seed, wherein the *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 is either a female parent or a male parent in a cross-fertilization.
25. A cell of an F1 hybrid plant grown from the F1 hybrid seed produced by the use of claim 24.
26. A cell of an F1 hybrid plant grown from the F1 hybrid seed produced by a method comprising crossing a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 with a different *Brassica carinata* plant and harvesting the resultant F1 hybrid carinata seed.
27. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 to produce a Double Haploid.
28. A cell, seed, plant or plant part of a Double Haploid produced by the use of claim 27.
29. Use of a *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 to produce a *Brassica carinata* plant comprising a desired trait, wherein the desired trait is introduced by crossing the *Brassica carinata* plant produced from the cell of a *Brassica carinata* plant of any one of claims 1 through 7 with a plant of another *Brassica* species comprising the desired trait.