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(54) **Title:** MRP VARIANTS IN BRASSICA

(57) **Abstract:** Provided are plants, cells, tissues, and germplasm thereof comprising one or more targeted alterations of the genomic sequences of the *MRP1* or *MRP2* genes that encode for multidrug resistance-associated protein (*MRP*), as well as meal made from such plant materials. Also provided are methods and compositions to make altered *MRP1* and *MRP2* gene variants; breeding methods and methods of identifying and selecting plant materials having the disclosed *MRP1* and *MRP2* variants.

WO 2025/006547 A2

MRP VARIANTS IN BRASSICA

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The official copy of the sequence listing is submitted electronically as an xml-formatted sequence listing file named 96770-US-PSP_ST26 created on June 29, 2023, and having a size of 267,241 bytes which is filed concurrently with the specification. The sequence listing comprised in this xml-formatted document is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE DISCLOSURE

[0002] The disclosures relates to phosphorous metabolism in *Brassica*, variants of MRP genes that reduce phytate and increase inorganic phosphate in *Brassica* grain and meal made thereof.

BACKGROUND

[0003] *Brassica napus* (also referred to herein as canola or oilseed rape) is an allotetraploid (2n= 4x = 38, AACC) comprising two full genome sets, four component genomes, and total of 38 chromosomes. The A genome includes 10 chromosomes and is derived from *B. rapa* (2n = 2x = 20, AA). The C genome includes 9 chromosomes and is derived from *B. oleracea* (2n = 2x = 18, CC). *B. napus* is one of the most important vegetable oilseed crops in the world, especially in China, Canada, the European Union and Australia. Canola meal, the fraction of the seed remaining after crushing and oil extraction, is approximately 55% of the volume of canola seed.

[0004] While canola meal is rich in protein and capable of providing a substantial amount of energy when used in animal feed; it also includes levels of anti-nutritional factors such as glucosinolates, tannins, phytate (or phytic acid), and sinapine. Since meal comprises about half of the seed volume of canola, and the demand for canola/oilseed rape has risen and is expected to continue rising to meet demands for healthy cooking oils, biodiesel, and personal care products, there is a long-felt need to modify the compositional properties of canola meal and thereby increasing its nutritional value.

[0005] Animal growth and development require phosphorus. While plant-based animal feed such as canola meal contains a large amount of phosphorus, most of the phosphorus is in the form of phytate. Phytate sequesters phosphate in a form that prevents its absorption by monogastric animals such as pigs and poultry. To supply monogastric animal need for free, inorganic phosphorus, plant-based meals are often supplemented with dicalcium/monocalcium phosphate or

treatment with a phytase enzyme that converts phytate to usable inorganic phosphate (Pi). Such supplementation adds to the cost and steps of preparing a diet for the animals. Accordingly there is a desire for new varieties of canola that contain higher levels of inorganic phosphate (Pi) in the seeds/grain used for feed formulation. This would reduce or eliminate the need for dicalcium/monocalcium phosphate or phytase supplements, and thereby reduce the cost of feed for animal production.

[0006] Biosynthetic pathway genes involved in phytate production and transport have been identified in the model plant *Arabidopsis* and multiple crop plants including *AtITPK1*, *AtITPK2*, *OsMIPS*, *OsIPK*, *OsMIK*, *OsMRP5*, *OsPLD1*, *OsLPA1*, *ZmMRP4*, *BnPGK2*, *BnITPK1* and *BnITPK2*. For example, maize *lpa1* gene (*ZmMRP4*) seed-specific silencing was found to reduce phytate and increase Pi in maize and a similar phenotype also was observed in transgenic soybean with the expression of homologous MRP genes silenced. See Shi et al., 2007 and US7511198B2. However, in *Brassica napus*, *BnA9.MRP5* was associated with reduced phytate inasmuch as *BnA9.MRP5* transcription levels were found to be "significantly elevated" relative to a high phytate variety. Liu et al. 2021 *Authorea*, DOI: 10.22541/au. 161165160.08519096/v1, see Abstract. In another study, double mutants of *BnMRP5* on chromosomes A10 and C09 showed "no significant differences in Pi" or inorganic phosphate. Sashidhar et al. 2020 *Frontiers in Plant Science*, 11 (Article 603): 1-10, at 1 and 5, 2nd col.

[0007] There is a desire to modify genes and develop new varieties of *Brassica napus* that not only provide reduced phytate content but also higher levels of inorganic phosphate (Pi).

SUMMARY OF THE DISCLOSURE

[0008] The disclosed compositions and methods are based, at least in part, on the surprising discovery that levels of inorganic phosphate in *Brassica napus* can be increased by a targeted alteration of the genomic sequences of *MRP1* and *MRP2* genes that encode for multidrug resistance-associated protein (*MRP*). The evidence herein demonstrates that targeted alterations of *MRP1*, *MRP2*, or both *MRP1* and *MRP2* lead to significantly increased levels of free or inorganic phosphate in *Brassica napus* plants. The term *Brassica napus* as used herein includes crop varieties of the species such as spring oilseed rape, winter oilseed rape, and low erucic cultivars of the foregoing which are called canola.

[0009] Provided herein is a method of increasing inorganic phosphate in *Brassica napus* plant, cell, seed, tissue or germplasm thereof, that comprises introducing a targeted alteration to the

sequence of one or more multidrug resistance-associated protein (*MRP*) genes. Targeted alterations can be made to an *MRP1* gene, to an *MRP2* gene, or to genes of both *MRP1* and *MRP2* to thereby generate a *Brassica napus* plant, cell, seed, tissue or germplasm thereof that comprises one or more *MRP* variants which can provide an increased level of inorganic phosphate relative to the plant, seed, tissue or germplasm thereof prior to introducing the one or more *MRP* variants. In one aspect, the method comprises introducing a targeted alteration of one or more alleles of *MRP2* on chromosome A09 or C09. In another aspect, the method comprises introducing a targeted alteration of one or more alleles of *MRP1* on chromosome A10 or C05. In yet another aspect, the method comprises introducing a targeted alteration of one or more alleles of *MRP2* and a targeted alteration of one or more alleles of *MRP1*. The types of targeted alterations that can be used to create *MRP* variants disclosed herein include nonsense mutations, missense mutations, and deletions that eliminate or reduce *MRP* gene function. For example, the targeted alteration can be a premature termination codon that reduces or eliminates expression of a full-length protein encoded by the altered *MRP* variant. Particular examples of a targeted alteration that introduces a premature termination codon are shown in Table 2 for *MRP2* on chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for *MRP1* on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP2.C05), and Table 4 for MRP2.A09 or MRP2.C09. Examples of a targeted alteration comprising a premature termination codon is shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43.

[0010] Disclosed herein is a method of increasing inorganic phosphate in *Brassica napus* plant, cell, seed, tissue or germplasm thereof, that comprises introducing a targeted alteration to the sequence of the multidrug resistance-associated protein (*MRP*) genes *MRP1* or *MRP2* to generate a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous variants of *MRP1*, *MRP2*, or a combination thereof. For example, targeted alterations can be introduced to make *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous knockouts of *MRP2* (null for both MRP2.A09 and MRP2.C09). In another example, targeted alterations can be introduced to make *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous knockouts of *MRP1* (null for both MRP1.A10

and MRP1.C05). In yet another example. targeted alterations can be introduced to make *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous knockouts of *MRP2* (null for both MRP2.A09 and MRP2.C09) and homozygous knockout alleles of one *MRP1* gene (null for MRP1.A10 or MRP1.C05). In still another example. targeted alterations can be introduced to make *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous knockouts of *MRP1* (null for both MRP1.A10 and MRP1.C05) and homozygous knockout alleles of one *MRP2* gene (null for MRP2.A09 or MRP2.C09). In one more example, targeted alterations can be introduced to make *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising double homozygous knockouts of both *MRP2* (null for both MRP2.A09 and MRP2.C09) and *MRP1* (null for both MRP1.A10 and MRP1.C05). Particular examples of such targeted alterations that can be used to generate two multidrug resistance-associated protein (*MRP*) gene alleles *MRP1* or *MRP2* are shown in Table 2 for *MRP2* on chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for *MRP1* on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP1.C05), and Table 4 for MRP2.A09 or MRP2.C09. Additionally examples of targeted alterations that can be used are shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43.

[0011] In particular examples, the disclosed method comprises introducing a targeted alteration into each of two *MRP* genes of *Brassica napus* plant, cell, seed, tissue or germplasm thereof, thereby introducing two *MRP* variant alleles, which are disclosed by reference to the variant alleles disclosed in Table 2, Table 3, or Table 4 herein, such that the term "MRP2.A9 Variant" refers to MRP2.A9_v1, MRP2.A9_v2, MRP2.A9_v3, MRP2.A9_v4, or MRP2.A9_v5, MRP2.A9_v6, MRP2.A9_v7, MRP2.A9_v8; the term "MRP2.C9 Variant" refers to MRP2.C9_v1, MRP2.C9_v2, MRP2.C9_v3, MRP2.C9_v4, MRP2.C9_v5, MRP2.C9_v6, MRP2.C9_v7; the term "MRP1.A10 Variant" refers to MRP1.A10_v1, MRP1.A10_v2, or MRP1.A10_v3; the term "MRP1.C05 Variant" refers to MRP1.C05_v1 or MRP1.C05_v2. For example, the method includes introducing (i) homozygous MRP2.A9_v1 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.A9_v2 combined with a heterozygous or homozygous MRP2.C9 Variant,

MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.A9_v3 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.A9_v4 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.A9_v5 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.A9_v6 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vii) homozygous MRP2.A9_v7 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (viii) homozygous MRP2.A9_v8 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant.

[0012] In additional examples, the disclosed method comprises introducing targeted alterations that generate a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising (i) homozygous MRP2.C9_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.C9_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.C9_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.C9_v4 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.C9_v5 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.C9_v6 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (vii) homozygous MRP2.C9_v7 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant.

[0013] In still other examples, the disclosed method comprises introducing targeted alterations that generate a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising (i) homozygous MRP1.A10_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; (ii) homozygous MRP1.A10_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; or (iii) homozygous MRP1.A10_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant. In still another example ; provided herein is a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising (i) homozygous MRP1.C05_v1

combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant; or homozygous MRP1.C05_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant.

[0014] In each of the foregoing methods of introducing targeted alterations to one or more *MRP* gene alleles, the *Brassica napus* plant, cell, seed, tissue or germplasm thereof that is altered can also further comprise an altered or variant gene that provides additional desirable meal quality. Thus, the method can comprise introducing targeted alterations that generate one or more *MRP* variants in *Brassica napus* plant, cell, seed, tissue or germplasm thereof that further includes a targeted alteration, mutation or variant of one or more of *LPA1, TT2, TT8, DFR, F3H, ANR, LDOX, MYB28* or *MAM1*. The desirable meal quality provided by *LPA1* is reduced phytate and/or increased inorganic phosphate content. Each of *TT2, TT8, DFR, and F3H, ANR,* and *LDOX* can reduce fiber content. Each of *MYB28* and *MAM1* can reduce glucosinolate content. The method can also comprise introducing targeted alterations that generate one or more *MRP* variants in *Brassica napus* plant, cell, seed, tissue or germplasm thereof that provides increased *Brassica napus* meal protein and/or low fiber. Such germplasm, for example, are disclosed in US Patent Nos. 9,375,025 and 10,791,692; as well as International Patent Application Publication Nos. WO 2020/131600.

[0015] Also provided herein are *Brassica napus* plant materials produced by targeted alteration or ethyl methanesulfonate (EMS) mutagenesis. Accordingly, provided herein is a *Brassica napus* plant, cell, seed, tissue or germplasm thereof that comprises one or more *MRP* variants, wherein the variants comprise a targeted alteration or an EMS mutant of *MRP1*, *MRP2*, or both *MRP1* and *MRP2*. *MRP* variants can comprise (i) a targeted alteration or EMS mutant of one or more alleles of *MRP2* on chromosome A09 or C09, (ii) a targeted alteration or EMS mutant of one or more alleles of *MRP1* on chromosome A10 or C05, or (iii) a combination of both (i) and (ii). The types of targeted alterations and EMS mutants include nonsense mutations, missense mutations, and deletions that eliminate or reduce *MRP* gene function. For example, the targeted alteration can be a premature termination codon that reduces or eliminates expression of a full-length protein encoded by the altered *MRP* allele. Particular examples of a targeted alteration that introduces a premature termination codon are shown in Table 2 for *MRP2* on chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for *MRP1* on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP2.C05), and Table 4 for MRP2.A09 or MRP2.C09. An example of a

targeted alteration comprising a premature termination codon is shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. An example of an EMS mutant is shown in each of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83.

[0016] In other examples, the disclosure provides *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising any combination of *MRP* variant alleles disclosed herein including (see definition above of terms "MRP2.A9 Variant", "MRP2.C9 Variant," "MRP1.A10 Variant", or "MRP1.C05 Variant" and variants disclosed in Table 2, Table 3, or Table 4 herein): (i) homozygous MRP2.A9_v1 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.A9_v2 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.A9_v3 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.A9_v4 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.A9_v5 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.A9_v6 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vii) homozygous MRP2.A9_v7 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (viii) homozygous MRP2.A9_v8 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant. (ix) homozygous MRP2.C9_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (x) homozygous MRP2.C9_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xi) homozygous MRP2.C9_v3 combined with a heterozygous or homozygous MRP2.A9 Variant,

MRP1.A10 Variant, or MRP1.C05 Variant; (xii) homozygous MRP2.C9_v4 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiii) homozygous MRP2.C9_v5 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiv) homozygous MRP2.C9_v6 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (xv) homozygous MRP2.C9_v7 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xvi) homozygous MRP1.A10_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; (xvii) homozygous MRP1.A10_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; or (xviii) homozygous MRP1.A10_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant. In still another example; the *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprises (i) homozygous MRP1.CO5_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant; or homozygous MRP1.CO5_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant.

[0017] In other examples, the disclosure provides *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising any combination of *MRP* EMS mutants alleles disclosed herein. As used herein an "MRP2.C9 EMS mutant" refers to an MRP2 variant carrying the mutation shown in any one of SEQ ID NOs:58-67; "MRP1.A10 EMS mutant" refers to an MRP variant carrying the mutation shown in any one of SEQ ID NOs:68-74; and "MRP1.C5 EMS mutant" refers to an MRP mutant carrying the mutation shown in any one of SEQ ID NOs:75-83. Thus, provided herein is *Brassica napus* plant, cell, seed, tissue or germplasm thereof that comprises: (1) any one or more of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83, (2) homozygous MRP2.C9 EMS mutant combined with heterozygous or homozygous MRP1.A10 EMS mutant or MRP1.C5 EMS mutant, (3) homozygous MRP1.A10 EMS mutant combined with heterozygous or homozygous MRP2.C9

EMS or MRP1.C5 EMS mutant, or (4) homozygous MRP1.C5 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.A10 EMS mutant.

[0018] In particular examples, each of the foregoing *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising one or more *MRP* variants can further include a targeted alteration, mutation or variant of one or more of the following genes: *LPA1, TT2, TT8, DFR, F3H, ANR, LDOX, MYB28* or *MAM1*, which can provide additional desirable meal quality. In additional examples, each of the foregoing *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising one or more *MRP* variants can further include increased *Brassica napus* meal protein and/or low fiber. Such germplasm, for example, are disclosed in US Patent Nos. 9,375,025 and 10,791,692; as well as International Patent Application Publication Nos. WO 2020/131600.

[0019] Each of the *Brassica napus* plant, seed, tissue or germplasm thereof comprising one or more *MRP* variants disclosed herein can be used to produce oilseed or grain which is milled to produce meal, e.g., canola meal. In preferred embodiments, the meal produced from the disclosed *Brassica napus* oilseed comprises increased levels of inorganic phosphate relative to control seed or grain lacking the one or more *MRP* variants. As used herein control refers to seed or grain that lacks the one or more *MRP* variants but is otherwise isogenic or substantially isogenic to the *Brassica napus* disclosed herein.

[0020] Accordingly provided herein is a method of producing high inorganic phosphate Brassica napus meal, the method comprising providing or selecting any of the *Brassica napus* seed or grain disclosed herein that comprises one or more *MRP* variants, wherein the variants comprise a targeted alteration of *MRP1*, *MRP2*, or both *MRP1* and *MRP2* and the selected seed or grain comprise increased inorganic phosphate relative to control seed or grain lacking the one or more MRP variants. The method then comprises milling the selected seed or grain to produce high inorganic phosphate *Brassica napus* meal. In any of the aspects, embodiments or examples of this method disclosed herein, the method can further include providing this high inorganic phosphate *Brassica napus* meal in feed to an animal, e.g., a monogastric animal. Preferred monogastric animals include swine and chickens (e.g., egg-laying hens, broilers, pullets, etc.) as well as other poultry such as turkeys, ducks, geese, guinea fowl, etc. Feed containing this high inorganic phosphate *Brassica napus* meal can also be fed to horses, rabbit, or any other commercially raised animal.

[0021] In one aspect, a method of producing high inorganic phosphate *Brassica napus* meal comprises milling *Brassica napus* seed or grain that comprises a targeted alteration to one or more alleles of *MRP2* on chromosome A09 or C09 or a targeted alteration to one or more alleles of *MRP1* on chromosome A10 or C05. The types of targeted alterations include nonsense mutations, missense mutations, and deletions that eliminate or reduce *MRP* gene function. For example, the targeted alteration can be a premature termination codon that reduces or eliminates expression of a full-length protein encoded by the altered *MRP* allele. Particular examples of a targeted alteration that introduces a premature termination codon are shown in Table 2 for *MRP2* on chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for *MRP1* on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP2.C05), and Table 4 for MRP2.A09 or MRP2.C09. An example of a targeted alteration comprising a premature termination codon is shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. In other examples of this method of producing high inorganic phosphate meal, the method comprises milling seed or grain that comprises any combination of *MRP* variants disclosed herein (see definition above of terms "MRP2.A9 Variant", "MRP2.C9 Variant," "MRP1.A10 Variant", or "MRP1.C05 Variant" and variants disclosed in Table 2, Table 3, or Table 4 herein): (i) homozygous MRP2.A9_v1 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.A9_v2 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.A9_v3 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.A9_v4 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.A9_v5 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.A9_v6 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vii) homozygous MRP2.A9_v7 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (viii) homozygous MRP2.A9_v8 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant. (ix) homozygous MRP2.C9_v1

combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (x) homozygous MRP2.C9_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xi) homozygous MRP2.C9_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xii) homozygous MRP2.C9_v4 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiii) homozygous MRP2.C9_v5 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiv) homozygous MRP2.C9_v6 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (xv) homozygous MRP2.C9_v7 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xvi) homozygous MRP1.A10_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; (xvii) homozygous MRP1.A10_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; or (xviii) homozygous MRP1.A10_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant. In still another example; the *Brassica napus* seed or grain used to produce high inorganic phosphate meal comprises (i) homozygous MRP1.CO5_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant; or homozygous MRP1.CO5_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant.

[0022] In another aspect, a method of producing high inorganic phosphate *Brassica napus* meal comprises milling *Brassica napus* seed or grain that comprises one or more mutant alleles of *MRP2* on chromosome A09 or C09 or one or more mutant alleles of *MRP1* on chromosome A10 or C05. Thus, the method comprises milling seed or grain that comprises any combination of *MRP* EMS mutants disclosed herein (see definition above of terms "MRP2.C9 EMS mutant", "MRP1.A10 EMS mutant", and "MRP1.C5 EMS mutant") that comprises: (1) any one or more of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81,

SEQ ID NO:82, or SEQ ID NO:83, (2) homozygous MRP2.C9 EMS mutant combined with heterozygous or homozygous MRP1.A10 EMS mutant or MRP1.C5 EMS mutant, (3) homozygous MRP1.A10 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.C5 EMS mutant, or (4) homozygous MRP1.C5 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.A10 EMS mutant.

[0023] In another aspect, provided herein is a screening method for identifying *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising one or more *MRP* variants associated with increased inorganic phosphate level. The method comprises providing a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising one or more *MRP* variants disclosed herein (e.g., any of the foregoing disclosed examples of a *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising one or more *MRP* variants), obtaining a sample comprising nucleic acid from the plant, cell, seed, tissue or germplasm thereof, then screening the sample for any of the following: a) the one or more *MRP* variants or b) one or more marker alleles within 5 cM (of and genetically linked to the one or more of the *MRP* variants. The method can then further comprise detecting the one or more (i) *MRP* variants or (ii) marker alleles in the sample to thereby identify the *Brassica napus* plant, cell, seed, tissue or germplasm thereof as having an *MRP* variant associated with increased inorganic phosphate level.

[0024] In still further embodiments, the method can additionally include selecting the *Brassica napus* plant, cell, seed, tissue or germplasm thereof identified as having an *MRP* variant or *MRP* EMS mutant associated with increased inorganic phosphate level. The selected plant material can be used for breeding or trait introgression.

[0025] For example, the method of identifying can include screening the sample for the presence of a marker allele linked to the *MRP* variant, e.g., by 5 cM, 4 cM, 3 cM, 2 cM, 1 cM, 0.9 cM, 0.8 cM, 0.7 cM, 0.6 cM, 0.5 cM, 0.4 cM, 0.3 cM, 0.2 cM, 0.1 cM, or less on a single meiosis-based genetic map, and associated. In other examples, the method can include detecting one or more targeted alteration to one or more alleles of *MRP2* on chromosome A09 or C09 or a targeted alteration to one or more alleles of *MRP1* on chromosome A10 or C05. The types of targeted alterations include nonsense mutations, missense mutations, and deletions that eliminate or reduce *MRP* gene function (e.g., a premature termination codon that reduces or eliminates expression of a full-length protein encoded by the altered *MRP* allele). Particular examples of a targeted alteration that can be detected in accordance with the method are shown in Table 2 for *MRP2* on

chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for MRP1 on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP2.C05), and Table 4 for MRP2.A09 or MRP2.C09. The method can include detecting a premature termination codon is shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. In other examples, this method can include detecting any combination of MRP variants disclosed herein (see definition above of terms "MRP2.A9 Variant", "MRP2.C9 Variant," "MRP1.A10 Variant", or "MRP1.C05 Variant" and variants disclosed in Table 2, Table 3, or Table 4 herein): (i) homozygous MRP2.A9_v1 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.A9_v2 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.A9_v3 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.A9_v4 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.A9_v5 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.A9_v6 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vii) homozygous MRP2.A9_v7 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (viii) homozygous MRP2.A9_v8 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant. (ix) homozygous MRP2.C9_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (x) homozygous MRP2.C9_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xi) homozygous MRP2.C9_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xii) homozygous MRP2.C9_v4 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiii) homozygous MRP2.C9_v5 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiv) homozygous MRP2.C9_v6 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or

(xv) homozygous MRP2.C9_v7 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xvi) homozygous MRP1.A10_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; (xvii) homozygous MRP1.A10_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; or (xviii) homozygous MRP1.A10_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant. In still another example; the method can include detecting (i) homozygous MRP1.CO5_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant; or homozygous MRP1.CO5_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant.

[0026] In another example, the method of identifying can include screening the sample for the presence of a marker allele linked to an *MRP* EMS mutant, e.g., by 5 cM, 4 cM, 3 cM, 2 cM, 1 cM, 0.9 cM, 0.8 cM, 0.7 cM, 0.6 cM, 0.5 cM, 0.4 cM, 0.3 cM, 0.2 cM, 0.1 cM, or less on a single meiosis-based genetic map, and associated. In other examples, the method can include detecting one or more *MRP* EMS mutant of *MRP2* on chromosome A09 or C09 or a *MRP* EMS mutant of *MRP1* on chromosome A10 or C05. The method can include can include screening the sample for the presence of a marker allele linked to one or more of SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83. In other examples, this method can include detecting any combination of *MRP* EMS mutants disclosed herein (see definition above of terms “MRP2.A9 Variant”, “MRP2.C9 Variant,” “MRP1.A10 Variant”, or “MRP1.C05 Variant” and variants disclosed in Table 2, Table 3, or Table 4 herein), e.g., a combination of a (1) homozygous MRP2.C9 EMS mutant combined with heterozygous or homozygous MRP1.A10 EMS mutant or MRP1.C5 EMS mutant, (2) homozygous MRP1.A10 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.C5 EMS mutant, or (3) homozygous MRP1.C5 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.A10 EMS mutant.

[0027] In another aspect, disclosed herein is a method that includes crossing a *Brassica napus* plant disclosed herein comprising one or more *MRP* variants to a second plant that does not have the one or more *MRP* variants, thereby producing one or more progeny plants whose genome comprises the one or more *MRP* variants, i.e., any of the *MRP* variants or combinations disclosed herein and/or selected by the screening method disclosed herein. In a one example of this aspect, the second plant is one of a plant line (a "recurrent parent line") and the method further includes crossing the progeny plant with another plant of the recurrent parent line to produce a second-generation progeny whose genome comprises the one or more *MRP* variants. Optionally, the second-generation progeny can be crossed with the recurrent parent line to produce a third-generation progeny whose genome comprises the one or more *MRP* variants. This process can be repeated three, four, five, six, seven, or more times, such that each subsequent generation progeny is crossed with the recurrent parent line, thereby introgressing the one or more *MRP* variants into the recurrent parent line.

[0028] In an alternative method, a plant having the one or more *MRP* variants disclosed herein is crossed with a second plant to produce progeny plants. The progeny plants are screened for the one or more *MRP* variants in accordance with the screening method disclosed herein. Generally, such screening includes obtaining a nucleic acid sample from each of the progeny plants and screening the sample for the presence of *MRP* variants; thereby identifying novel progeny plants comprising a one of the R genes disclosed herein.

[0029] Provided herein is an isolated recombinant nucleic acid comprising one or more of SEQ ID NOs: 13-43 or SEQ ID NOs:53-67. In particular example, the isolated recombinant nucleic acid is a gene editing construct comprising a guide RNA (e.g., any one or more of SEQ ID NO:13, SEQ ID NO:16, or SEQ ID NO:19) for introducing an *MRP* variant disclosed herein. In another example, provided herein is a combination of primers for detecting an *MRP* variant disclosed herein, e.g., SEQ ID NO:14 and SEQ ID NO:15; or SEQ ID NO:17 and SEQ ID NO:18; or SEQ ID NO:20 and SEQ ID NO:21.

[0030] In another aspect, provided herein is a method of introducing an *MRP* variant into *Brassica napus*, the method comprising delivering to a cell or tissue of the *Brassica napus* a Cas endonuclease and a guide RNA targeting a sequence (target site) of *MRP2* gene on chromosome A09 or C09 or *MRP1* gene on chromosome A10 or C05. The endonuclease/guide RNA complex indues a targeted alteration at the target site which reduces or eliminates expression of the protein

encoded by the targeted *MRP2* or *MRP1* gene, thereby introducing an *MRP* variant to the *Brassica napus* cell or tissue. In some examples, the method further comprises regenerating *Brassica napus* plant, cell, seed, tissue or germplasm thereof comprising the *MRP* variant. The guide RNA can be any one or more of SEQ ID NO:13, SEQ ID NO:16, or SEQ ID NO:19. The *MRP* variant can be any *MRP* variant disclosed herein and shown in Table 2 for *MRP2* on chromosome A09 (MRP2.A09) or on chromosome C09 (MRP2.C09), Table 3 for MRP1 on chromosome A10 (MRP1.A10) or on chromosome C05 (MRP2.C05), and Table 4 for MRP2.A09 or MRP2.C09. Thus, the variant can include the premature termination codon shown in each of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. Additionally the method can include introducing any combination of MRP variant alleles disclosed herein (see definition above of terms "MRP2.A9 Variant", "MRP2.C9 Variant," "MRP1.A10 Variant", or "MRP1.C05 Variant" and variants disclosed in Table 2, Table 3, or Table 4 herein): (i) homozygous MRP2.A9_v1 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (ii) homozygous MRP2.A9_v2 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iii) homozygous MRP2.A9_v3 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (iv) homozygous MRP2.A9_v4 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (v) homozygous MRP2.A9_v5 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vi) homozygous MRP2.A9_v6 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (vii) homozygous MRP2.A9_v7 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (viii) homozygous MRP2.A9_v8 combined with a heterozygous or homozygous MRP2.C9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant. (ix) homozygous MRP2.C9_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (x) homozygous MRP2.C9_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xi) homozygous MRP2.C9_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or

MRP1.C05 Variant; (xii) homozygous MRP2.C9_v4 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiii) homozygous MRP2.C9_v5 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xiv) homozygous MRP2.C9_v6 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; or (xv) homozygous MRP2.C9_v7 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP1.A10 Variant, or MRP1.C05 Variant; (xvi) homozygous MRP1.A10_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; (xvii) homozygous MRP1.A10_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant; or (xviii) homozygous MRP1.A10_v3 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.C05 Variant. In still another example; the method can include introducing (i) homozygous MRP1.CO5_v1 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant; or homozygous MRP1.CO5_v2 combined with a heterozygous or homozygous MRP2.A9 Variant, MRP2.C9 Variant, or MRP1.A10 Variant.

[0031] In another aspect, provided herein is a method of introducing an *MRP* variant into *Brassica napus* to generate any one of the mutations shown in Table 5 herein within SEQ ID NOs:53-83, the method comprising delivering to a cell or tissue of the *Brassica napus* a Cas endonuclease and a guide RNA targeting a sequence (target site) of *MRP2* gene on chromosome A09 or C09 or *MRP1* gene on chromosome A10 or C05. The method can include generating several such variants such that the Cas endonuclease and guide RNA produce changes leading to a combination of the mutations shown in SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83, such that the variant comprises the mutations of a (1) homozygous MRP2.C9 EMS mutant combined with heterozygous or homozygous MRP1.A10 EMS mutant or MRP1.C5 EMS mutant, (2) homozygous MRP1.A10 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.C5 EMS

mutant, or (3) homozygous MRP1.C5 EMS mutant combined with heterozygous or homozygous MRP2.C9 EMS or MRP1.A10 EMS mutant.

[0032] Methods for introducing Cas endonucleases and guide RNAs are described in more detail herein.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

[0033] Fig. 1 is a bar graph showing levels of inorganic phosphate (Pi) in seed comprising double homozygous knockout of MRP2.A9/MRP2.C9 and control seed comprising respective wild type *MRP2* genes.

[0034] Fig. 2 is a bar graph showing Pi levels of seed comprising double homozygous knockout of MRP1.A10/MRP1.C05 and control seed comprising respective wild type *MRP1* genes.

[0035] Fig. 3 is a bar graph showing Pi levels of seed comprising triple homozygous knockout alleles of MRP2.A9/MRP2.C9/MRP2.C9a and control seed comprising respective wild type *MRP2* genes.

[0036] Fig. 4 is a bar graph showing Pi levels of hybrid seed comprising double homozygous knockout of MRP2.A9/MRP2.C9 and control hybrid seed comprising respective wild type *MRP2* genes.

[0037] Fig. 5 is a bar graph showing Pi levels of inbred seed harvested from field trials in 2021, one set of seed comprised double homozygous knockout of MRP2.A9/MRP2.C9 and the control set hybrid seed comprised respective wild type *MRP2* genes.

[0038] Fig. 6 is a bar graph showing Pi levels of seeds in the following lines harvested from field trials in 2022: double homozygous knockout of *MRP2* in G00010BC inbred and corresponding control (wild type *MRP2* genes) G00010BC inbred; double homozygous knockout of *MRP2* in G00555MC and corresponding control (wild type *MRP2* genes) G00555MC inbred; double homozygous knockout of *MRP2* in second generation F2 hybrid seed and corresponding control (wild type *MRP2* genes) hybrid.

[0039] Fig. 7 is a bar graph showing Pi levels of seed in *MRP2/TT2* double knockout variants and corresponding control wild type plants.

[0040] Fig. 8 is a bar graph showing ADF levels of meal in *MRP2/TT2* double knockout variants and corresponding control wild type plants.

[0041] Nucleic acid sequences listed in the accompanying sequence listing and referenced herein are shown using standard letter abbreviations for nucleotide bases. While only one strand of each nucleic acid sequence is shown, the complementary strand is understood to be included in any reference to the displayed strand. Sequence listings are described in the following Table 1 and in Table 5 herein.

Table 1

Sequence	Description
SEQ ID NO:1	Genomic sequence MRP2 chromosome A9
SEQ ID NO:2	Coding sequence MRP2 chromosome A9
SEQ ID NO:3	Encoded protein MRP2 chromosome A9
SEQ ID NO:4	Genomic sequence MRP2 chromosome C9
SEQ ID NO:5	Coding sequence MRP2 chromosome C9
SEQ ID NO:6	Encoded protein MRP2 chromosome C9
SEQ ID NO:7	Genomic sequence MRP1 chromosome A10
SEQ ID NO:8	Coding sequence MRP1 chromosome A10
SEQ ID NO:9	Encoded protein MRP1 chromosome A10
SEQ ID NO:10	Genomic sequence MRP1 chromosome C5
SEQ ID NO:11	Coding sequence MRP1 chromosome C5
SEQ ID NO:12	Encoded protein MRP1 chromosome C5
SEQ ID NO:13	GGTACAGGGTCGTGCTTGA MRP2-CR1 guide RNA
SEQ ID NO:14	TTCTGAAACTGCAGGCTTGG MRP2-CR1 Forward primer
SEQ ID NO:15	GCTACGAAGATAGGCGAACTCC MRP2-CR1 Reverse primer
SEQ ID NO:16	GGCTTGGGAGGATCGGTAC MRP2-CR2 guide RNA
SEQ ID NO:17	GGAGTGTTTGAGGAACATGAGG MRP2-CR2 Forward primer
SEQ ID NO:18	GGGAGTATAAAGCCTTTCGAAGC MRP2-CR2 Reverse primer
SEQ ID NO:19	CACGAAGGATACGTCCTCG MRP1-CR1 guide RNA
SEQ ID NO:20	GCTGGCTTGAACACTCTCTTATCC MRP1-CR1 Forward primer
SEQ ID NO:21	ACCCCCATGTACCACTGACG MRP1-CR1 Reverse primer
SEQ ID NO:22	Genomic sequence MRP2.A9_v1 variant
SEQ ID NO:23	Genomic sequence MRP2.A9_v2 variant
SEQ ID NO:24	Genomic sequence MRP2.A9_v3 variant
SEQ ID NO:25	Genomic sequence MRP2.A9_v4 variant
SEQ ID NO:26	Genomic sequence MRP2.A9_v5 variant
SEQ ID NO:27	Genomic sequence MRP2.C9_v1 variant
SEQ ID NO:28	Genomic sequence MRP2.C9_v2 variant
SEQ ID NO:29	Genomic sequence MRP1.A10_v1 variant
SEQ ID NO:30	Genomic sequence MRP1.A10_v2 variant
SEQ ID NO:31	Genomic sequence MRP1.A10_v3 variant
SEQ ID NO:32	Genomic sequence MRP1.C5_v1 variant
SEQ ID NO:33	Genomic sequence MRP1.C5_v2 variant
SEQ ID NO:34	Genomic sequence MRP2.A9_v6 variant
SEQ ID NO:35	Genomic sequence MRP2.A9_v7 variant
SEQ ID NO:36	Genomic sequence MRP2.A9_v8 variant

SEQ ID NO:37	Genomic sequence MRP2.C9a_v3 variant
SEQ ID NO:38	Genomic sequence MRP2.C9a_v4 variant
SEQ ID NO:39	Genomic sequence MRP2.C9a_v5 variant
SEQ ID NO:40	Genomic sequence MRP2.C9a_v6 variant
SEQ ID NO:41	Genomic sequence MRP2.C9a_v7 variant
SEQ ID NO:42	Genomic sequence MRP2.C9b_v8 variant
SEQ ID NO:43	Genomic sequence MRP2.C9b_v9 variant
SEQ ID NO:44	G00555MC genomic sequence MRP2 chromosome A9
SEQ ID NO:45	G00555MC coding sequence MRP2 chromosome A9
SEQ ID NO:46	G00555MC encoded protein MRP2 chromosome A9
SEQ ID NO:47	G00555MC genomic sequence MRP2 chromosome C9a
SEQ ID NO:48	G00555MC coding sequence MRP2 chromosome C9a
SEQ ID NO:49	G00555MC encoded protein MRP2 chromosome C9a
SEQ ID NO:50	G00555MC genomic sequence MRP2 chromosome C9b
SEQ ID NO:51	G00555MC coding sequence MRP2 chromosome C9b
SEQ ID NO:52	G00555MC encoded protein MRP2 chromosome C9b

DETAILED DESCRIPTION

[0042] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the protein" includes reference to one or more proteins and equivalents thereof, and so forth. 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 disclosure belongs unless clearly indicated otherwise.

[0043] A gene or allele is "associated with" a trait when it is part of or linked to a DNA sequence or allele that affects the expression of the trait. The presence of the allele is an indicator of how the trait will be expressed.

[0044] *"Brassica"* refers to any one of *Brassica napus* (AACC, 2n=38), *Brassica juncea* (AABB, 2n=36), *Brassica carinata* (BBCC, 2n= 34), *Brassica rapa* (syn. B. campestris) (AA, 2n=20), *Brassica oleracea* (CC, 2n=18) or *Brassica nigra* (BB, 2n= 16).

[0045] "Backcrossing" refers to the process whereby hybrid progeny are repeatedly crossed back to one of the parents. In a backcrossing scheme, the "donor" parent refers to the parental plant with the desired gene or locus to be introgressed. The "recipient" parent (used one or more times) or "recurrent" parent (used two or more times) refers to the parental plant into which the gene or locus is being introgressed.

[0046] "CRISPR" (Clustered Regularly Interspaced Short Palindromic Repeats) loci refers to certain genetic loci encoding components of DNA cleavage systems, for example, used by bacterial and archaeal cells to destroy foreign DNA (Horvath and Barrangou, 2010, *Science* 327:167-170; International Application Publication WO2007/025097, published 01 March 2007). A CRISPR locus can consist of a CRISPR array, comprising short direct repeats (CRISPR repeats) separated by short variable DNA sequences (called spacers), which can be flanked by diverse Cas (CRISPR-associated) genes.

[0047] The term "Cas protein" refers to a polypeptide encoded by a Cas (CRISPR-associated) gene. A Cas protein includes but is not limited to: a Cas9 protein, a Cpf1 (Cas12) protein, a C2c1 protein, a C2c2 protein, a C2c3 protein, Cas3, Cas3-HD, Cas 5, Cas7, Cas8, Cas10, or combinations or complexes of these. A Cas protein may be a "Cas endonuclease" or "Cas effector protein", that when in complex with a suitable polynucleotide component, is capable of recognizing, binding to, and optionally nicking or cleaving all or part of a specific polynucleotide target sequence. A Cas endonuclease described herein comprises one or more nuclease domains. The endonucleases of the disclosure may include those having one or more RuvC nuclease domains. A Cas protein is further defined as a functional fragment or functional variant of a native Cas protein, or a protein that shares at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with a native Cas protein, and retains at least partial activity.

[0048] A "Cas endonuclease" may comprise domains that enable it to function as a double-strand-break-inducing agent. A "Cas endonuclease" may also comprise one or more modifications or mutations that abolish or reduce its ability to cleave a double-strand polynucleotide (dCas). In some aspects, the Cas endonuclease molecule may retain the ability to nick a single-strand polynucleotide (for example, a D10A mutation in a Cas9 endonuclease molecule) (nCas9).

[0049] The term "crossed" or "cross" refers to a sexual cross and involved the fusion of two haploid gametes via pollination to produce diploid progeny (e.g., cells, seeds or plants). The term encompasses both the pollination of one plant by another and selfing (or self-pollination, e.g., when the pollen and ovule are from the same plant).

[0050] An "elite line" is any line that has resulted from breeding and selection for superior agronomic performance.

[0051] A "favorable allele" is the allele at a particular locus (a marker, a QTL, a gene etc.) that confers, or contributes to, an agronomically desirable phenotype, e.g., disease resistance, and that allows the identification of plants with that agronomically desirable phenotype. A favorable allele of a marker is a marker allele that segregates with the favorable phenotype.

[0052] "Gene" includes a nucleic acid fragment that expresses a functional molecule such as, but not limited to, a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence, as well as intervening intron sequences. "Native gene" refers to a gene as found in its natural endogenous location with its own regulatory sequences.

[0001] "Genetic markers" are nucleic acids that are polymorphic in a population and where the alleles of which can be detected and distinguished by one or more analytic methods, e.g., RFLP, AFLP, isozyme, SNP, SSR, and the like. The term also refers to nucleic acid sequences complementary to the genomic sequences, such as nucleic acids used as probes. Markers corresponding to genetic polymorphisms between members of a population can be detected by methods well-established in the art. These include, e.g., PCR-based sequence specific amplification methods, detection of restriction fragment length polymorphisms (RFLP), detection of isozyme markers, detection of polynucleotide polymorphisms by allele specific hybridization (ASH), detection of amplified variable sequences of the plant genome, detection of self-sustained sequence replication, detection of simple sequence repeats (SSRs), detection of single nucleotide polymorphisms (SNPs), or detection of amplified fragment length polymorphisms (AFLPs). Well established methods are also known for the detection of expressed sequence tags (ESTs) and SSR markers derived from EST sequences and randomly amplified polymorphic DNA (RAPD).

[0053] "Germplasm" refers to genetic material of or from an individual (e.g., a plant), a group of individuals (e.g., a plant line, variety or family), or a clone derived from a line, variety, species, or culture, or more generally, all individuals within a species or for several species (e.g., maize germplasm collection or Andean germplasm collection). The germplasm can be part of an organism, cell, or can be separate from the organism or cell. In general, germplasm provides genetic material with a specific molecular makeup that provides a physical foundation for some or all of the hereditary qualities of an organism or cell culture. As used herein, germplasm includes cells, seed or tissues from which new plants may be grown, or plant parts, such as leaves, stems, pollen, or cells, that can be cultured into a whole plant.

[0054] The term "genome" as it applies to a prokaryotic and eukaryotic cell or organism cells encompasses not only chromosomal DNA found within the nucleus, but organelle DNA found within subcellular components (e.g., mitochondria, or plastid) of the cell.

[0055] As used herein, a "genomic sequence" or "genomic region" is a segment of a chromosome in the genome of a cell that is present on either side of the target site or, alternatively, also comprises the target site or a portion thereof. An "endogenous genomic sequence" refers to genomic sequence within a plant cell, (e.g. an endogenous genomic sequence of an *MRP* gene present within the genome of a *Brassica* plant cell).

[0056] A "genomic locus" as used herein refers to the genetic or physical location on a chromosome of a gene. As used herein, "gene" includes a nucleic acid fragment that expresses a functional molecule such as, but not limited to, a specific protein coding sequence and regulatory elements, such as those preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence.

[0057] As used herein, "genotype" is the actual nucleic acid sequence at one or more loci in an individual plant. As used herein, "phenotype" means the detectable characteristics (e.g. increased free phosphate) of a cell or organism which can be influenced by genotype.

[0058] As used herein, the term "guide polynucleotide", relates to a polynucleotide sequence that can form a complex with a Cas endonuclease, including the Cas endonuclease described herein, and enables the Cas endonuclease to recognize, optionally bind to, and optionally cleave a DNA target site. The guide polynucleotide sequence can be a RNA sequence, a DNA sequence, or a combination thereof (a RNA-DNA combination sequence).

[0059] The terms "single guide RNA" and "sgRNA" are used interchangeably herein and relate to a synthetic fusion of two RNA molecules, a crRNA (CRISPR RNA) comprising a variable targeting domain (linked to a tracr mate sequence that hybridizes to a tracrRNA), fused to a tracrRNA (trans-activating CRISPR RNA). The single guide RNA can comprise a crRNA or crRNA fragment and a tracrRNA or tracrRNA fragment of the type II CRISPR/Cas system that can form a complex with a type II Cas endonuclease, wherein said guide RNA/Cas endonuclease complex can direct the Cas endonuclease to a DNA target site, enabling the Cas endonuclease to recognize, optionally bind to, and optionally nick or cleave (introduce a single or double-strand break) the DNA target site.

[0060] As used herein, the terms "guide polynucleotide/Cas endonuclease complex", "guide polynucleotide/Cas endonuclease system", " guide polynucleotide/Cas complex", "guide polynucleotide/Cas system" and "guided Cas system" "Polynucleotide-guided endonuclease" , "PGEN" are used interchangeably herein and refer to at least one guide polynucleotide and at least one Cas endonuclease, that are capable of forming a complex, wherein said guide polynucleotide/Cas endonuclease complex can direct the Cas endonuclease to a DNA target site, enabling the Cas endonuclease to recognize, bind to, and optionally nick or cleave (introduce a single or double-strand break) the DNA target site. A guide polynucleotide/Cas endonuclease complex herein can comprise Cas protein(s) and suitable polynucleotide component(s) of any of the known CRISPR systems (Horvath and Barrangou, 2010, Science 327:167-170; Makarova et al. 2015, Nature Reviews Microbiology Vol. 13:1-15; Zetsche et al., 2015, Cell 163, 1-13; Shmakov et al., 2015, Molecular Cell 60, 1-13).

[0061] A "haplotype" is the genotype of an individual at a plurality of genetic loci, i.e. a combination of alleles. Typically, the genetic loci described by a haplotype are physically and genetically linked, i.e., on the same chromosome segment.

[0062] The term "heterogeneity" is used to indicate that individuals within the group differ in genotype at one or more specific loci.

[0063] The term "homogeneity" indicates that members of a group have the same genotype at one or more specific loci.

[0064] The term "hybrid" refers to the progeny obtained between the crossing of at least two genetically dissimilar parents.

[0065] The term "inbred" refers to a line that has been bred for genetic homogeneity.

[0066] The term "inorganic phosphate" and "free phosphate" are used interchangeably herein to mean forms of nutritional phosphate that can be absorbed by monogastric animals such as pigs and poultry.

[0067] The term "introgression" refers to the transmission of a desired allele of a genetic locus from one genetic background to another. For example, introgression of a desired R gene allele at a specified locus can be transmitted to at least one progeny via a sexual cross between two parents of the same species, where at least one of the parents has the desired allele in its genome. Alternatively, for example, transmission of an allele can occur by recombination between two donor genomes, e.g., in a fused protoplast, where at least one of the donor protoplasts has the

desired allele in its genome. The desired allele can be, e.g., detected by a marker that is associated with a phenotype, at a QTL, a transgene, or the like. Offspring comprising the desired allele may be repeatedly backcrossed to a line having a desired genetic background and selected for the desired allele, to result in the allele becoming fixed in a selected genetic background.

[0068] The process of "introgressing" is often referred to as "backcrossing" when the process is repeated two or more times.

[0069] A "line" or "strain" is a group of individuals of identical parentage that are generally inbred to some degree and that are generally homozygous and homogeneous at most loci (isogenic or near isogenic). A "subline" refers to an inbred subset of descendants that are genetically distinct from other similarly inbred subsets descended from the same progenitor.

[0070] The term "plant material" includes whole plants, plant cells, plant protoplast, plant cell or tissue culture from which plants can be regenerated, plant calli, plant clumps and plant cells that are intact in plants, or parts of plants, such as seeds, flowers, cotyledons, leaves, stems, buds, roots, root tips and the like. As used herein, a "modified plant" means any plant that has a genetic change due to human intervention. A modified plant may have genetic changes introduced through plant transformation, genome editing, mutagenesis, or conventional plant breeding.

[0071] A "marker" is a means of finding a position on a genetic or physical map, or else linkages among markers and trait loci (loci affecting traits). The position that the marker detects may be known via detection of polymorphic alleles and their genetic mapping, or else by hybridization, sequence match or amplification of a sequence that has been physically mapped. A marker can be a DNA marker (detects DNA polymorphisms), a protein (detects variation at an encoded polypeptide), or a simply inherited phenotype (such as a low-erucic acid oil profile). A DNA marker can be developed from genomic nucleotide sequence or from expressed nucleotide sequences (e.g., from a spliced RNA or a cDNA). Depending on the DNA marker technology, the marker may consist of primers complementary to sequence flanking the locus and/or probes that hybridize to polymorphic alleles at the locus. A DNA marker, or a genetic marker, may also be used to describe the gene, DNA sequence or nucleotide on the chromosome itself (rather than the components used to detect the gene or DNA sequence) and is often used when that DNA marker is associated with a particular trait in human genetics (e.g. a marker for breast cancer). The term marker locus is the locus (gene, sequence or nucleotide) that the marker detects.

[0072] Markers can be defined by the type of polymorphism that they detect and also the marker technology used to detect the polymorphism. Marker types include but are not limited to, e.g., detection of restriction fragment length polymorphisms (RFLP), detection of isozyme markers, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), detection of simple sequence repeats (SSRs), detection of amplified variable sequences of the plant genome, detection of self-sustained sequence replication, or detection of single nucleotide polymorphisms (SNPs). SNPs can be detected e.g. via DNA sequencing, PCR-based sequence specific amplification methods, detection of polynucleotide polymorphisms by allele specific hybridization (ASH), dynamic allele-specific hybridization (DASH), molecular beacons, microarray hybridization, oligonucleotide ligase assays, Flap endonucleases, 5' endonucleases, primer extension, single strand conformation polymorphism (SSCP) or temperature gradient gel electrophoresis (TGGE). DNA sequencing, such as the pyrosequencing technology has the advantage of being able to detect a series of linked SNP alleles that constitute a haplotype. Haplotypes tend to be more informative (detect a higher level of polymorphism) than SNPs.

[0073] "Marker assisted selection" (of MAS) is a process by which individual plants are selected based on marker genotypes. "Marker assisted counter-selection" is a process by which marker genotypes are used to identify plants that will not be selected, allowing them to be removed from a breeding program or planting. A "marker haplotype" refers to a combination of alleles at a marker locus.

[0074] The term "molecular marker" may be used to refer to a genetic marker, as defined above, or an encoded product thereof (e.g., a protein) used as a point of reference when identifying a linked locus. A molecular marker can be derived from genomic nucleotide sequences or from expressed nucleotide sequences (e.g., from a spliced RNA, a cDNA, etc.), or from an encoded polypeptide. The term also refers to nucleic acid sequences complementary to or flanking the marker sequences, such as nucleic acids used as probes or primer pairs capable of amplifying the marker sequence. A "molecular marker probe" is a nucleic acid sequence or molecule that can be used to identify the presence of a marker locus, e.g., a nucleic acid probe that is complementary to a marker locus sequence. Alternatively, in some aspects, a marker probe refers to a probe of any type that is able to distinguish (i.e., genotype) the particular allele that is present at a marker locus. Nucleic acids are "complementary" when they specifically hybridize in solution. Some of the

markers described herein are also referred to as hybridization markers when located on an indel region, such as the non-collinear region described herein. This is because the insertion region is, by definition, a polymorphism *vis a vis* a plant without the insertion. Thus, the marker need only indicate whether the indel region is present or absent. Any suitable marker detection technology may be used to identify such a hybridization marker, e.g. SNP technology is used in the examples provided herein.

[0075] As used herein, a 'nucleic acid molecule" is a polymeric form of nucleotides, which can include both sense and anti-sense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. A nucleotide refers to a ribonucleotide, deoxynucleotide, or a modified form of either type of nucleotide. A "nucleic acid molecule" as used herein is synonymous with "nucleic acid", "nucleotide sequence", "nucleic acid sequence", and "polynucleotide." The term includes single- and double-stranded forms of DNA. A nucleic acid molecule can include either or both naturally occurring and modified nucleotides linked together by naturally occurring and/or non-naturally occurring nucleotide linkages.

[0076] Nucleic acid molecules may be modified chemically or biochemically, or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications, such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., peptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). The term "nucleic acid molecule" also includes any topological conformation, including single-stranded, double-stranded, partially duplexed, triplexed, hairpinned, circular, and padlocked conformations. An "endogenous nucleic acid sequence" refers to a nucleic acid sequence within a plant cell, (e.g. an endogenous allele of an *IND* gene present within the genome of a *Brassica* plant cell).

[0077] A "protospacer adjacent motif" (PAM) herein refers to a short nucleotide sequence adjacent to a target sequence (protospacer) that is recognized (targeted) by a guide polynucleotide/Cas endonuclease system described herein. The Cas endonuclease may not successfully recognize a target DNA sequence if the target DNA sequence is not followed by a PAM sequence. The sequence and length of a PAM herein can differ depending on the Cas protein

or Cas protein complex used. The PAM sequence can be of any length but is typically 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nucleotides long.

[0078] As used herein, the term "plant material" refers to any processed or unprocessed material derived, in whole or in part, from a plant. For example, and without limitation, a plant material may be a plant part, a seed, a fruit, a leaf, a root, a plant tissue, a plant tissue culture, a plant explant, or a plant cell.

[0079] A "polymorphism" is a variation in the DNA between two or more individuals within a population. A polymorphism preferably has a frequency of at least 1% in a population. A useful polymorphism can include a single nucleotide polymorphism (SNP), a simple sequence repeat (SSR), or an insertion/deletion polymorphism, also referred to herein as an "indel".

[0080] "Phytate" and "phytic acid" are used interchangeably herein to mean forms of inositol polyphosphate (inositol hexakisphosphate, IP6, dihydrogenphosphate ester of inositol) which sequester their constituent phosphate and inhibit its absorption when consumed by monogastric animals such as pigs and poultry.

[0081] The term "quantitative trait locus" or "QTL" refers to a region of DNA that is associated with the differential expression of a quantitative phenotypic trait in at least one genetic background, e.g., in at least one breeding population. The region of the QTL encompasses or is closely linked to the gene or genes that affect the trait in question.

[0082] The terms "target site", "target sequence", "target site sequence, "target DNA", "target locus", "genomic target site", "genomic target sequence", "genomic target locus", and "target polynucleotide", can be used interchangeably herein and refer to a polynucleotide sequence such as, but not limited to, a nucleotide sequence on a chromosome, episome, a locus, or any other DNA molecule in the genome (including chromosomal, chloroplastic, mitochondrial DNA, plasmid DNA) of a cell, at which a guide polynucleotide/Cas endonuclease complex can recognize, bind to, and optionally nick or cleave . The target site can be an endogenous site in the genome of a cell, or alternatively, the target site can be heterologous to the cell and thereby not be naturally occurring in the genome of the cell, or the target site can be found in a heterologous genomic location compared to where it occurs in nature. As used herein, terms "endogenous target sequence" and "native target sequence" are used interchangeable herein to refer to a target sequence that is endogenous or native to the genome of a cell and is at the endogenous or native position of that target sequence in the genome of the cell.

[0083] A "targeted alteration" or "variant" is a gene (e.g., *MRP* gene) sequence that has been altered through human intervention. Such an "altered" or "modified" gene has a sequence that differs from the sequence of the corresponding native or non-altered gene by at least one nucleotide (i) insertion (i.e., addition of a nucleotide in a sequence), (ii) deletion, (iii) substitution (i.e., replacement of at least one nucleotide), or (iv) a combination of the foregoing alterations. An "altered" or "modified" plant is a plant comprising an altered gene sequence, e.g., a deletion. As used herein, a "targeted alteration" in a gene (referred to as the target gene) can be made by altering a target sequence within the target gene using any method known to one skilled in the art, including a method involving a guided Cas endonuclease system as disclosed herein.

[0084] A virus or vector "transforms" or "transduces" a cell when it transfers nucleic acid molecules into the cell. A cell is "transformed" by a nucleic acid molecule transduced into the cell when the nucleic acid molecule becomes stably replicated by the cell, either by incorporation of the nucleic acid molecule into the cellular genome, or by episomal replication. As used herein, the term "transformation" encompasses all techniques by which a nucleic acid molecule can be introduced into such a cell. Examples include, but are not limited to, transfection with viral vectors, transformation with plasmid vectors, electroporation (Fromm et al., 1986, *Nature* 319:791-3), lipofection (Feigner et al., 1987, *Proc. Natl. Acad. Sci. USA* 84:7413-7), microinjection (Mueller et al., 1978, *Cell* 15:579-85), *Agrobacterium*-mediated transfer (Fraley et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:4803-7), direct DNA uptake, and microprojectile bombardment (Klein et al., 1987, *Nature* 327:70).

[0085] The term "variants" refer to substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more internal sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or more sites in the native polynucleotide (e.g. an *MRP* variant disclosed herein). As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively.

[0086] The term "yield" refers to the productivity per unit area of a particular plant product of commercial value. Yield is affected by both genetic and environmental factors. "Agronomics," "agronomic traits," and "agronomic performance" refer to the traits (and underlying genetic elements) of a given plant variety that contribute to yield over the course of growing season. Individual agronomic traits include emergence vigor, vegetative vigor, stress tolerance, disease

resistance or tolerance, herbicide resistance, branching, flowering, seed set, seed size, seed density, standability, threshability and the like. Yield can therefore be considered the final culmination of all agronomic traits.

[0087] The disclosed targeted alterations or variants of an MRP gene refer to human-made, intentionally produced and selected nucleic acid changes that can be generated by any known methods, including the use of targeted mutagenesis, Targeting Induced Local Lesions IN Genomes or TILLING (see e.g., McCallum et al., 2000, *Nat Biotechnol* 18:455-457), or the use of double-strand-break inducing agents (DSB Agents).

[0088] Double-Strand-Break (DSB) Inducing Agents (DSB Agents). Double-strand breaks can be induced by agents such as endonucleases that cleave the phosphodiester bond within a polynucleotide chain, can result in the induction of DNA repair mechanisms, including the non-homologous end-joining pathway, and homologous recombination. Endonucleases include a range of different enzymes, including restriction endonucleases (*see e.g.* Roberts et al., 2003 *Nucleic Acids Res* 1:418-20, Roberts et al., 2003, *Nucleic Acids Res* 31:1805-12, and Belfort et al., 2002 in *Mobile DNA II*, pp. 761-783, Eds. Craigie et al., (ASM Press, Washington, DC)), meganucleases (*see e.g.,* International Application Publication WO 2009/114321; Gao et al., 2010, *Plant Journal* 1:176-187), and TAL effector nucleases or TALENs (*see e.g.*, US Application Publication US 20110145940 and Christian et al., 2010, *Genetics* 186(2): 757-61). Methods of targeting DNA double-strand breaks have been described for TALENs (Christian et al., 2010, *Genetics* 186(2): 757-61 and Boch et al., 2009, Science 326(5959):1509-12), zinc finger nucleases (see e.g. Kim, et al., 1996, *Proc. Nat'l Acad. Sci USA* 93(3)1156-1160) and CRISPR-Cas endonucleases (see e.g. International Application Publication WO2007/025097).

[0089] Any DSB or -nick or -modification inducing agent may be used for the methods described herein, including for example but not limited to: Cas endonucleases, recombinases, TALENs, zinc finger nucleases, restriction endonucleases, meganucleases, and deaminases.

[0090] Methods and compositions are provided for polynucleotide modification with a CRISPR Associated (Cas) endonuclease. Class I Cas endonucleases comprise multi-subunit effector complexes (Types I, III, and IV), while Class 2 systems comprise single protein effectors (Types II, V, and VI) (Makarova et al., 2015, *Nature Reviews Microbiology* 13:1-15; Zetsche et al., 2015, *Cell* 163:1-13; Shmakov et al., 2015, *Molecular Cell* 60, 1-13; Haft et al., 2005, *Computational Biology, PLoS Comput Biol 1*(6): e60; and Koonin et al., 2017, *Curr Opinion*

Microbiology 37:67-78). In Class 2 Type II systems, the Cas endonuclease acts in complex with a guide RNA (gRNA) that directs the Cas endonuclease to cleave the DNA target to enable target recognition, binding, and cleavage by the Cas endonuclease. The gRNA comprises a Cas endonuclease recognition (CER) domain that interacts with the Cas endonuclease, and a Variable Targeting (VT) domain that hybridizes to a nucleotide sequence in a target DNA. In some aspects, the gRNA comprises a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA) to guide the Cas endonuclease to its DNA target. The crRNA comprises a spacer region complementary to one strand of the double strand DNA target and a region that base pairs with the tracrRNA, forming an RNA duplex. In many systems, the Cas endonuclease-guide polynucleotide complex recognizes a short nucleotide sequence adjacent to the target sequence (protospacer), called a "protospacer adjacent motif" (PAM).

[0091] Examples of a Cas endonuclease include but are not limited to Cas9, Cas12f, Cas12a or Cpf1, and variants thereof (See e.g., US Patent No. 10,934,536 and International Application Publication WO 2022/082179). Cas9 (formerly referred to as Cas5, Csn1, or Csx12) is a Class 2 Type II Cas endonuclease (Makarova et al., 2015, *Nature Reviews Microbiology* 13:1-15). For Cas9 and Cas12f, a Cas-gRNA complex recognizes a 3' PAM sequence at the target site, permitting the spacer of the guide RNA to invade the double-stranded DNA target, and, if sufficient homology between the spacer and protospacer exists, generate a DSB cleavage. Cas9 endonucleases comprise RuvC and HNH domains that together produce DSBs, and separately can produce single strand breaks. For the *S. pyogenes* Cas9 endonuclease, the DSB leaves a blunt end. Cpf1 is a Class 2 Type V Cas endonuclease, and comprises nuclease RuvC domain but lacks an HNH domain (Yamane et al., 2016, *Cell* 165:949-962). Cas12f can generate 5' staggered overhangs at DSB sites (Karvelis et al., *Nucl Acids Res* 48(12):5016-5023). Cpf1 endonucleases create "sticky" overhang ends.

[0092] Some uses for Cas-gRNA systems at a genomic target site include but are not limited to insertions, deletions, substitutions, or modifications of one or more nucleotides at the target site; modifying or replacing nucleotide sequences of interest (such as a regulatory elements); insertion of polynucleotides of interest; gene dropout; gene knock-out; gene knock in; modification of splicing sites and/or introducing alternate splicing sites; modifications of nucleotide sequences encoding a protein of interest; amino acid and/or protein fusions; and gene silencing by expressing an inverted repeat into a gene of interest. Genome editing using DSB-inducing agents, such as

Cas9-gRNA complexes, has been described, for example in U.S. Patent Application No. 2015/0082478, US Patent No. 10,934,536, International Application Publication WO2015/026886 A1, International Application Publication WO2016007347, International Application Publication WO201625131, and International Application Publication WO 2022/082179 all of which are incorporated by reference herein.

[0093] In some aspects of the disclosure, a targeted genomic modification is introduced in a *B. napus* plant cell, wherein the targeted modification includes a targeted alteration of the genomic sequence of an *MRP* gene in the *B. napus* plant cell. In a further aspect, the targeted genomic modification is induced by a DSB Agent, such as a CRISPR-associated (Cas) nuclease. A Cas nuclease is introduced into the *B. napus* cell with a first and second guide RNAs as Cas-gRNA complexes that recognizes target sequences in the genome of the *B. napus* cell and is able to induce DSBs in the genomic sequence, e.g., thereby altering the endogenous target *MRP* gene.

[0094] Recombinant Constructs and Transformation of Cells. The disclosed guide polynucleotides can be introduced into a cell with the disclosed DSB agents e.g., CRISPR-Cas endonucleases. Cells include, but are not limited to, human, non-human, animal, bacterial, fungal, insect, yeast, non-conventional yeast, and plant cells as well as plants and seeds produced by the methods described herein. In a preferred aspect of the disclosure, the cells are *B. napus* cells.

[0095] Standard recombinant DNA and molecular cloning techniques used herein are known in the art and are described more fully in Sambrook et al., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY (1989). Transformation methods are well known to those skilled in the art and are described *infra*.

[0096] Vectors and constructs include circular plasmids, and linear polynucleotides, comprising a polynucleotide of interest and optionally other components including linkers, adapters, regulatory or analysis. In some examples a recognition site and/or target site can be comprised within an intron, coding sequence, 5' UTRs, 3' UTRs, and/or regulatory regions.

[0097] In one aspect, the constructs of the disclosure comprise a promoter operably linked to a nucleotide sequence encoding a DSB Agent, such as a CAS nuclease (e.g., gene encoding a *Streptococcus pyrogenes* Cas9 gene or Cas12f gene) and a promoter operably linked to a guide RNA of the present disclosure. The promoter is capable of driving expression of an operably linked nucleotide sequence in a prokaryotic or eukaryotic cell/organism. In some aspects, target specific

guide RNAs are built as a fusion of CRISPR RNA (crRNA) fused to trans-activating CRISPR RNA (tracrRNA) of *Streptococcus pyrogenes*.

[0098] In accordance with the methods disclosed herein, a guide RNA comprising SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, can be used to alter endogenous genomic *MRP* sequence in the *B. napus* plant cell. The resulting targeted alterations can produce an *MRP* variant comprising a premature termination codon as shown in any of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43.

[0099] The isolated polynucleotides, constructs and vectors disclosed herein (e.g., for expression of endonucleases or guide RNAs) can comprise a selectable marker to identify or select for or against a molecule or a cell that comprises the construct or vector. Examples of selectable markers that can be used in a construct or vector disclosed herein include *DsRed* and *Glyphosate N-Acetyltransferase* (*GAT*) gene variant 4621 for herbicide resistance.

[0100] Isolated Nucleic Acid Molecules and Variants and Fragments Thereof. Isolated or recombinant nucleic acid molecules comprising *MRP* variants disclosed herein as well as active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify *MRP* variants by sequence homology are provided. As used herein, the term "nucleic acid molecule" refers to DNA molecules (e.g., recombinant DNA, cDNA, genomic DNA, plastid DNA, mitochondrial DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. In some examples, the nucleic acid molecule can be single-stranded. In some examples, the nucleic acid molecule can be double-stranded.

[0101] An "isolated" nucleic acid molecule (e.g., RNA or DNA) is used herein to refer to a nucleic acid sequence (e.g., RNA or DNA) that is no longer in its natural environment, for example *in vitro*. A "recombinant" nucleic acid molecule (e.g., RNA or DNA) is used herein to refer to a nucleic acid sequence (e.g., RNA or DNA) that is in a recombinant bacterial or plant host cell; has been edited from its native sequence; or is located in a different location than the native sequence. In some embodiments, an "isolated" or "recombinant" nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5′ and 3′ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid

is derived. For purposes of the disclosure, "isolated" or "recombinant" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the recombinant nucleic acid molecules can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleic acid sequences that naturally flank the *MRP* variant in the genome of the cell.

[0102] In some embodiments, an isolated nucleic acid molecule comprising an *MRP* variant has one or more change in the nucleic acid sequence compared to the native or genomic nucleic acid sequence. In some embodiments, the change in the native or genomic nucleic acid sequence includes but is not limited to: changes in the nucleic acid sequence due to the degeneracy of the genetic code; changes in the nucleic acid sequence due to the amino acid substitution, insertion, deletion and/or addition compared to the native or genomic sequence; removal of one or more intron; deletion of one or more upstream or downstream regulatory regions; and deletion of the 5' and/or 3' untranslated region associated with the genomic nucleic acid sequence. In some embodiments, the nucleic acid molecule comprising one of SEQ ID NOs:13-43 or SEQ ID NOs:53-67is non-genomic sequence.

[0103] A variety of polynucleotides comprising *MRP* variants disclosed herein are contemplated. Such polynucleotides are useful for production of encoded polypeptides in host cells when operably linked to a suitable promoter, transcription termination and/or polyadenylation sequences. Such polynucleotides are also useful as probes for isolating homologous or substantially homologous polynucleotides that are *MRP* variants or related to *MRP* variants disclosed herein.

[0104] Provided herein are nucleic acid molecules comprising one or more of SEQ ID NOs:13-43 or SEQ ID NOs:58-67, and variants, fragments and complements thereof. "Complement" is used herein to refer to a nucleic acid sequence that is sufficiently complementary to a given nucleic acid sequence such that it can hybridize to the given nucleic acid sequence to thereby form a stable duplex. A reverse complement is a complement formed by exchanging each A with T, T with A, C with G, and G with C in a sequence and then reversing the 5' to 3' order of the exchanged sequence, such that the reverse complement of 5'-ACCTGAG-3' is 5'-CTCAGGT-3'. "Polynucleotide sequence variants" is used herein to refer to a nucleic acid sequence that except for the degeneracy of the genetic code encodes the same polypeptide.

[0105] "Percent (%) sequence identity" with respect to a reference sequence (subject) is determined as the percentage of amino acid residues or nucleotides in a candidate sequence (query)

that are identical with the respective amino acid residues or nucleotides in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any amino acid conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent sequence identity can be achieved in various ways, for instance, using publicly available computer software such as BLAST, BLAST-2. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (e.g., percent identity of query sequence = number of identical positions between query and subject sequences/total number of positions of query sequence ×100).

[0106] Nucleotide Constructs, Expression Cassettes and Vectors. The use of the term "construct" in connection with isolated and/or heterologous polynucleotides herein is not intended to limit the disclosure to constructs comprising DNA. Polynucleotide constructs, particularly polynucleotides and oligonucleotides composed of ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides, may also be employed in the methods disclosed herein. The isolated polynucleotide constructs, nucleic acids, and nucleotide sequences disclosed herein additionally encompass all complementary forms (e.g., the reverse complement) of each sequence disclosed for such a construct. Further, polynucleotide constructs and nucleotide sequences disclosed herein can encompass any such constructs, molecules, and sequences suitable for use in a method for transforming plant material disclosed herein. Such constructs can include naturally occurring molecules and/or synthetic analogues. The disclosed nucleotide constructs, nucleic acids, and nucleotide sequences also encompass all forms of nucleotide constructs including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures and the like.

[0107] Transformed organisms disclosed herein include plant cells, bacteria, yeast, baculovirus, protozoa, nematodes and algae. The transformed organism comprises a disclosed sequence (e.g., as part of a construct, expression cassette, or vector comprising the nucleotide sequence disclosed herein which are associated with increased disease resistance.

[0108] The disclosed sequences can be used in constructs for expression in the organism of interest. Constructs can include 5' and 3'; regulatory sequences operably linked to an R gene

sequence, variant or fragment disclosed herein. The term "operably linked" as used herein refers to a functional linkage between a promoter and/or a regulatory sequence and a second sequence, wherein the promoter and/or regulatory sequence initiates, mediates, and/or affects transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary, to join two protein coding regions in the same reading frame. The construct may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple DNA constructs.

[0109] Such a DNA construct is provided with a plurality of restriction sites for insertion of the polypeptide gene sequence of the disclosure to be under the transcriptional regulation of the regulatory regions. The DNA construct may additionally contain selectable marker genes.

[0110] The DNA construct will generally include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region (e.g., a promoter), a DNA sequence of the embodiments, and a transcriptional and translational termination region (e.g., termination region) functional in the organism serving as a host. The transcriptional initiation region (e.g., the promoter) may be native, analogous, foreign or heterologous to the host organism and/or to the sequence of the embodiments. Additionally, the promoter or regulatory sequence may be the natural sequence or alternatively a synthetic sequence. The term "foreign" as used herein indicates that the promoter is not found in the native organism into which the promoter is introduced. As used herein, the term "heterologous" in reference to a sequence means a sequence that originates from a foreign species or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence. Where the promoter is a native or natural sequence, the expression of the operably linked sequence is altered from the wild-type expression, which results in an alteration in phenotype.

[0111] In some embodiments the DNA construct comprises a polynucleotide comprising one or more of SEQ ID NOs:13-43 or SEQ ID NOs:58-67 or a fragment or variant thereof.

[0112] A DNA construct may also include a transcriptional enhancer sequence. An "enhancer" refers to a DNA sequence which can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a

promoter. Various enhancers include, for example, introns with gene expression enhancing properties in plants (US Patent Application Publication Number 2009/0144863, the ubiquitin intron (i.e., the maize ubiquitin intron 1 (see, for example, NCBI sequence S94464)), the omega enhancer or the omega prime enhancer (Gallie et al. 1989 *Molecular Biology of RNA* ed. Cech (Liss, New York) 237-256 and Gallie et al. 1987 *Gene* 60: 217-25), the CaMV 35S enhancer (see, e.g., Benfey et al. 1990 *EMBO J.* 9:1685-96) and the enhancers of US Patent Number 7,803,992 may also be used. The above list of transcriptional enhancers is not meant to be limiting. Any appropriate transcriptional enhancer can be used in the embodiments.

[0113] The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host or may be derived from another source (i.e., foreign or heterologous to the promoter, the sequence of interest, the plant host or any combination thereof).

[0114] Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau et al. 1991 *Mol. Gen. Genet.* 262:141-144; Proudfoot 1991 *Cell* 64:671-674; Sanfacon et al. 1991 *Genes Dev.* 5:141-149; Mogen et al. 1990 *Plant Cell* 2:1261-1272; Munroe et al. 1990 *Gene* 91:151-158; Ballas et al. 1989 *Nucleic Acids Res.* 17:7891-7903 and Joshi et al. 1987 *Nucleic Acid Res.* 15:9627-9639.

[0115] Where appropriate, a nucleic acid may be optimized for increased expression in the host organism. Thus, where the host organism is a plant, the synthetic nucleic acids can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri 1990 *Plant Physiol.* 92:1-11 for a discussion of host-preferred usage. For example, although nucleic acid sequences of the embodiments may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray et al. 1989 *Nucleic Acids Res.* 17:477-498). Thus, the plant-preferred for a particular amino acid may be derived from known gene sequences from plants.

[0116] Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other well-characterized sequences that may be deleterious to gene expression. The GC content of the sequence may be adjusted to levels

average for a given cellular host, as calculated by reference to known genes expressed in the host cell. The term "host cell" as used herein refers to a cell which contains a vector and supports the replication and/or expression of the expression vector is intended. Host cells may be prokaryotic cells such as *E. coli* or eukaryotic cells such as yeast, insect, amphibian or mammalian cells or monocotyledonous or dicotyledonous plant cells. An example of a monocotyledonous host cell is a maize host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

[0117] In preparing the expression cassette, the various DNA fragments may be manipulated so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

[0118] A number of promoters can be used in the practice of the embodiments. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, inducible

[0119] Plant Transformation. Plant Transformation. Any suitable techniques known in the art for introduction of transgenes into plants may be used to produce a transformed plant or plant cell disclosed herein. Suitable methods for transformation of plants may include virtually any method by which DNA can be introduced into a cell, such as: by electroporation as illustrated in U.S. Patent No. 5,384,253; by microprojectile bombardment, as illustrated in U.S. Patent Nos. 5,015,580, 5,550,318, 5,538,880, 6,160,208, 6,399,861, and 6,403,865; by *Agrobacterium*-mediated transformation as illustrated in U.S. Patent Nos. 5,635,055, 5,824,877, 5,591, 616; 5,981,840, and 6,384,301; and by protoplast transformation, as set forth in U.S. Patent No. 5,508,184, etc. These techniques can be used to transform plant cells and these cells may be developed into transgenic plants by techniques known to those of skill in the art. Techniques for transforming *Brassica plants* in particular are disclosed, for example, in U.S. Patent No. 5,750,871.

[0120] After effecting delivery of exogenous DNA to recipient cells, transformed cells are identified for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable marker gene with the transformation vector

used to generate the transformant. In this case, the potentially transformed cell population can be assayed by exposing the cells to a selective agent or agents, or the cells can be screened for the desired marker.

[0121] Cells that survive the exposure to the selective agent, or cells that have been scored positive in a screening assay, may be cultured in media that supports regeneration of plants. In some embodiments, any suitable plant tissue culture media may be modified by including further substances, such as growth regulators. Tissue may be maintained on a basic media with growth regulators until sufficient tissue is available to begin plant regeneration efforts, or following repeated rounds of manual selection, until the morphology of the tissue is suitable for regeneration (e.g., at least 2 weeks), then transferred to media conducive to shoot formation. Cultures are transferred periodically until sufficient shoot formation has occurred. Once shoots are formed, they are transferred to media conducive to root formation. Once sufficient roots are formed, plants can be transferred to soil for further growth and maturity.

[0122] The alteration (e.g., introduction of a stop codon, mutation or deletion) of an endogenous gene (e.g., *MRP1* or *MRP2*) in regenerating plants can be confirmed by one or more assays, for example, a molecular biological assay, such as Southern blotting, Northern blotting, or PCR; a biochemical assay, such as detecting the absence of a protein product by immunoassay (ELISA or Western blot) or by screening for reduced enzymatic function; plant part assays, such as leaf or root assays; and analysis of the phenotype of the whole regenerated plant.

[0123] Using the methods disclosed herein, *MRP* variant-containing plants are generated. For example, a plant comprising an altered endogenous genomic sequence containing one or more of the following *MRP* variants: SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43. In some examples, the modified *B. napus* plant comprises double homozygous variants of *MRP1, MRP2,* or both *MRP1* and *MRP2*.

[0124] "Stable transformation" as used herein means that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" as used herein means that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a

plant. "Plant" as used herein refers to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells and pollen).

[0125] Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway et al. (1986) *Biotechniques* 4:320-334), electroporation (Riggs et al. 1986 *Proc. Natl. Acad. Sci. USA* 83:5602-5606), *Agrobacterium*-mediated transformation (US Patent Numbers 5,563,055 and 5,981,840), direct gene transfer (Paszkowski et al. 1984 *EMBO J.* 3:2717-2722) and ballistic particle acceleration (see, for example, US Patent Numbers 4,945,050; 5,879,918; 5,886,244 and 5,932,782; Tomes et al. 1995 in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin) and McCabe et al. 1988 *Biotechnology* 6:923-926) and Lecl transformation (WO 00/28058). For potato transformation see, Tu et al. 1998 *Plant Molecular Biology* 37:829-838 and Chong et al. 2000 *Transgenic Research* 9:71-78. Additional transformation procedures can be found in Weissinger et al. 1988 *Ann. Rev. Genet.* 22:421-477; Sanford et al. 1987 *Particulate Science and Technology* 5:27-37 (onion); Christou et al. 1988 *Plant Physiol.* 87:671-674 (soybean); McCabe et al. 1988 *Bio/Technology* 6:923-926 (soybean); Finer and McMullen 1991 *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh et al. 1998 *Theor. Appl. Genet.* 96:319-324 (soybean); Datta et al. 1990 *Biotechnology* 8:736-740 (rice); Klein et al. 1988 *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein et al. 1988 *Biotechnology* 6:559-563 (maize); US Patent Numbers 5,240,855; 5,322,783 and 5,324,646; Klein et al. (1988) *Plant Physiol.* 91:440-444 (maize); Fromm et al. 1990 *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren et al. 1984 *Nature (London)* 311:763-764; US Patent Number 5,736,369 (cereals); Bytebier et al. 1987 *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet et al. 1985 in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman et al. (Longman, New York), pp. 197-209 (pollen); Kaeppler et al. 1990 *Plant Cell Reports* 9:415-418 and Kaeppler et al. 1992 *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin et al. 1992 *Plant Cell* 4:1495-1505 (electroporation); Li et al. (1993) *Plant Cell Reports* 12:250-255 and Christou

and Ford 1995 *Annals of Botany* 75:407-413 (rice); Osjoda et al. 1996 *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*).

[0126] Marker assisted selection. Molecular markers can be used in a variety of plant breeding applications (e.g. see Staub et al. 1996 *Hortscience* 31:729-741; Tanksley 1983 *Plant Molecular Biology Reporter.* 1:3-8). One of the main areas of interest is to increase the efficiency of backcrossing and introgressing genes using marker-assisted selection (MAS). Thus, MAS can be used for backcrossing and introgressing the MRP variants disclosed herein.

[0127] A molecular marker that demonstrates linkage with a locus affecting a desired phenotypic trait provides a useful tool for the selection of the trait in a plant population. This is particularly true where the phenotype is hard to assay. Since DNA marker assays are less laborious and take up less physical space than field phenotyping, much larger populations can be assayed, increasing the chances of finding a recombinant with the target segment from the donor line moved to the recipient line. The closer the linkage, the more useful the marker, as recombination is less likely to occur between the marker and the gene causing the trait, which can result in false positives. Having flanking markers decreases the chances that false positive selection will occur as a double recombination event would be needed. In the most preferred case, a marker is located within the gene itself, so that recombination cannot occur between the marker and the gene. In some embodiments, the methods targeted alteration disclosed herein produce a marker that can be used to identify the *MRP* variant.

[0128] Gene introgression by MAS can be used to diminish linkage drag and yield drag. Gepts 2002 *Crop Sci*; 42:1780-1790; Young et al. 1998 *Genetics* 120:579-585; Tanksley et al. 1989 *Biotechnology* 7: 257-264. Markers disclosed herein, as well as other marker types such as SSRs and FLPs, can be used in marker assisted selection protocols. SSRs can be defined as relatively short runs of tandemly repeated DNA with lengths of 6 bp or less, which are often highly suited to mapping and MAS. Tautz 1989 *Nucleic Acid Research* 17: 6463-6471; Wang et al. 1994 *Theoretical and Applied Genetics*, 88: 1-6; Levinson and Gutman 1987 *Mol Biol Evol* 4: 203-221; Weber and May 1989 *Am J Hum Genet.* 44:388-396; Rafalski et al. 1996 Generating and using DNA markers in plants. In: *Non-mammalian genomic analysis: a practical guide.* Academic press. pp 75-135). FLP markers refer to fragment length polymorphisms that are in many ways similar to SSR markers, except that the region amplified by the primers is not typically a highly repetitive region. Bhattramakki et al. 2002 *Plant Mol Biol* 48, 539-547; Rafalski 2002b, supra.

[0129] SNP markers detect single base pair nucleotide substitutions, which can be assayed at an even higher level of throughput than SSRs, in so-called "ultra-high-throughput" fashion, as SNPs do not require large amounts of DNA and automation of the assay may be straight-forward. SNPs also have the promise of being relatively low-cost systems. These three factors together make SNPs highly attractive for use in MAS. Several methods are available for SNP genotyping, including but not limited to, hybridization, primer extension, oligonucleotide ligation, nuclease cleavage, minisequencing, and coded spheres. Such methods have been reviewed in: Gut 2001 *Hum Mutat* 17: 475-492; Shi 2001 *Clin Chem* 47: 164-172; Kwok 2000 *Pharmacogenomics* 1: 95-100; and Bhattramakki and Rafalski 2001 Discovery and application of single nucleotide polymorphism markers in plants. In: R. J. Henry, Ed, *Plant Genotyping: The DNA Fingerprinting of Plants*, CABI Publishing, Wallingford. A wide range of commercially available technologies utilize these and other methods to interrogate SNPs including Masscode.TM. (Qiagen), INVADER®. (Third Wave Technologies) and Invader PLUS®, SNAPSHOT®. (Applied Biosystems), TAQMAN®. (Applied Biosystems) and BEADARRAYS®. (Illumina).

[0130] In addition to SSR's, FLPs and SNPs, as described above, other types of molecular markers are also widely used, including but not limited to expressed sequence tags (ESTs), SSR markers derived from EST sequences, randomly amplified polymorphic DNA (RAPD), and other nucleic acid based markers. Isozyme profiles and linked morphological characteristics can, in some cases, also be indirectly used as markers. Even though they do not directly detect DNA differences, they are often influenced by specific genetic differences. However, markers that detect DNA variation are far more numerous and polymorphic than isozyme or morphological markers (Tanksley 1983 *Plant Molecular Biology Reporter* 1: 3-8).

[0131] The following are examples of specific embodiments of some aspects of the invention. The examples are offered for illustrative purposes only and are not intended to limit the scope of the invention in any way.

EXAMPLES

[0132] Example 1: Gene-edited *MRP2* knockouts.

[0133] CRISPR-Cas gene editing system was used to edit *MRP2* genes in Pioneer *Brassica napus* inbred line G00010BC, which contains two *MRP2* genes. One *MRP2* gene is located on chromosome A9 (MRP2.A9) (genomic: SEQ ID NO:1, coding: SEQ ID NO:2, or encoding a. a.: SEQ ID NO:3) and another one is located on chromosome C9 (MRP2.C9) (genomic: SEQ ID

NO:4, coding: SEQ ID NO:5, or encoding a. a.: SEQ ID NO:6). A guide RNA (SEQ ID NO:13) was designed to target the second exon of *MRP2* and induce a double stranded cut in the *MRP2* gene, leaving two free chromosomal ends. The plant's repair mechanisms can attempt to repair the double-strand DNA break by non-homologous end joining ("NHEJ"), which can result in nucleotide insertions and deletions (Indels) at the cleavage site. Among these variants, those that result in a coding sequence frameshift are referred to as knockouts or "KOs".

[0134] Epicotyl explants of canola inbred G00010BC were transformed with *Agrobacterium tumefasciens* carrying a CRISPR-Cas9 Ti plasmid for plant transformation as described in Chu et al. 2020, *Frontiers in Plant Science*, Vol. 11, Article 579524. Regenerated plantlets were screened using a NGS sequencing assay to obtain the knockout variants identified herein. Table 2 shows various mutations generated in the *MRP2* gene variants using the indicated gRNA sequence. In Table 2, a period indicates the double-stranded break point that was targeted; underlined residues represent nucleotide insertions; dashes represent nucleotide deletions.

Table 2

Sequence Listing	Sequence	Name of variant / mutation
SEQ ID NO:1 positions 2121-2156	AGGTACAGGGTCGTGCT.TGAGGGAATGAGGAGTACA	wild type MRP2.A9/ none
SEQ ID NO:22 positions 2121-2157	AGGTACAGGGTCGTGCTATGAGGGAATGAGGAGTACA	MRP2.A9_v1 / Insertion
SEQ ID NO:23 positions 2121-2157	AGGTACAGGGTCGTGCTCTGAGGGAATGAGGAGTACA	MRP2.A9_v2 / Insertion
SEQ ID NO:24 positions 2121-2157	AGGTACAGGGTCGTGCTTTGAGGGAATGAGGAGTACA	MRP2.A9_v3 / Insertion
SEQ ID NO:25 positions 2121-2155	AGGTACAGGGTCGTGC--.TGAGGGAATGAGGAGTACA	MRP2.A9_v4 / Deletion
SEQ ID NO:26 positions 2121-2154	AGGTACAGGGTCGTG--.TGAGGGAATGAGGAGTACA	MRP2.A9_v5 / Deletion
SEQ ID NO:4 position 2047-2082	CGGTACAGGGTCGTGCT.TGAGGGAATGAGGAGTACG	wild type MRP2.C9/ none
SEQ ID NO:27 positions 2047-2083	CGGTACAGGGTCGTGCTATGAGGGAATGAGGAGTACG	MRP2.C9_v1 / Insertion
SEQ ID NO:28 positions 2047-2083	CGGTACAGGGTCGTGCTTTGAGGGAATGAGGAGTACG	MRP2.C9_v2 / Insertion

[0135] Plants identified as positive T0 knockout plants were self-pollinated to produce T1 seed. T1 seed was planted in growth chamber and seedlings were genotyped with the same NGS

sequencing assay to identify MRP2.A9 and MRP2.C9 single homozygous and double homozygous plants as well as wildtype (WT) segregants. Because T-DNA from the plasmid was incorporated into the genome of T0 plants, PCR assays were used to screen for T1 plants ("clean" T1 plants) that were null homozygous segregants for the desired MRP2 variants and that were free of genome-editing reagent plasmids. Selected clean T1 plants were self-pollinated to generate T2 seeds.

[0136] The free phosphorous inorganic phosphate (Pi) content of T2 seeds was evaluated both in whole seeds or in ground, defatted meal made from the seeds. The method used for Pi determination was a modified version of the methods described in Chen et. al. 1956, *Analytical Chemistry*, 28: 1756-1758. MRP2.A9 single homozygotes and MRP2.C9 single homozygotes were each found to have higher Pi than WT in T2 seeds. However, levels were not as high as desired. Double homozygous knockouts (null for both MRP2.A9 and MRP2.C9) had higher Pi than single homozygotes. See Fig. 1, showing that the double homozygous knockouts contained 83.1 μmoles Pi per gram seeds, which is significantly higher than wild type. In fat-free canola meal made from the double homozygous knockouts, free Pi concentration was 137.6 μmole/g and Pi phosphorus (Pi-P) was 4.3 mg/g.

[0137] The foregoing shows the use of targeted alterations to generate examples of MRP2 variants, including stacks of two homozygous knockouts MRP2 variants, that provide increased levels of inorganic phosphate, relative to wild type controls.

[0138] Example 2: Gene-edited *MRP1* knockouts.

[0139] CRISPR-Cas gene editing system was used to edit *MRP1* genes in Pioneer *Brassica napus* inbred line G00010BC, which also contains two *MRP1* genes. One *MRP1* gene is located on chromosome A10 (MRP1.A10) (genomic: SEQ ID NO:7, coding: SEQ ID NO:8, or encoding a. a.: SEQ ID NO:9) and another one is located chromosome C5 (MRP1.C5) (genomic: SEQ ID NO:10, coding: SEQ ID NO:11, or encoding a. a.: SEQ ID NO:12). A guide RNA (SEQ ID NO:19) was designed to target an exon of *MRP1* gene.

[0140] Transformation of CRISPR-Cas gene editing system was done as in Example 1. Regenerated plantlets were screened using a NGS sequencing assay to obtain the knockout variants identified herein. Table 3 shows variants generated in the *MRP1* genes using the indicated gRNA sequence. In Table 3, a period indicates the double-stranded break point that was targeted; underlined residues represent nucleotide insertions; dashes represent nucleotide deletions.

Table 3

Sequence Listing	Sequence	Name of variant / mutation
SEQ ID NO:7 positions 1552-1585	TCACGAAGGATACGTCC.TCGCGGGGATCTTCTTT	wild type MRP1.A10/ none
SEQ ID NO:29 positions 1552-1586	TCACGAAGGATACGTCC**C**TCGCGGGGATCTTCTTT	MRP1.A10_v1 / Insertion
SEQ ID NO:30 positions 1552-1586	TCACGAAGGATACGTCC**T**TCGCGGGGATCTTCTTT	MRP1.A10_v2 / Insertion
SEQ ID NO:31 positions 1552-1584	TCACGAAGGATACGTCC–.CGCGGGGATCTTCTTT	MRP1.A10_v3 / Deletion
SEQ ID NO:10 positions 1491-1525	TCACGAAGGATACGTCC.TCGCGGGGATCTTCTTT	wild type MRP1.C5/ none
SEQ ID NO:32 positions 1491-1526	TCACGAAGGATACGTCC**A**TCGCGGGGATCTTCTTT	MRP1.C05_v1 / Insertion
SEQ ID NO:33 positions 1491-1526	TCACGAAGGATACGTCC**C**TCGCGGGGATCTTCTTT	MRP1.C05_v2 / Insertion

[0141] Plants identified as positive T0 knockout plants were self-pollinated to produce T1 seed. T1 plants were genotyped as described in Example 1 to identify MRP1.A10 and MRP1.C05 single homozygous knockout and double homozygous knockout plants as well as wildtype (WT) segregants. Because T-DNA from the plasmid was incorporated into the genome of T0 plants, PCR assays were used to screen for "clean" T1 plants that were null homozygous segregants for the desired MRP1 variants and that were free of genome-editing reagent plasmids. Selected clean T1 plants were self-pollinated to generate T2 seeds.

[0142] Free phosphorous (Pi) content of T2 seeds was evaluated as described in Example 1. Double homozygous knockouts (null for both MRP1.A10 and MRP1.C05) contained 18.3 µmoles Pi per gram of seed, which is higher than wild type content of 10.0 µmoles Pi per gram of seed. See Fig. 2.

[0143] The foregoing shows the use of targeted alterations to generate examples of MRP1 variants, including stacks of two homozygous knockouts MRP1 variants, that provide increased levels of inorganic phosphate, relative to wild type controls.

[0144] Example 3: Effect of *MRP* gene knockouts on germination.

[0145] To determine if knocking out *MRP* gene affects seed germination, T2 seeds were placed in a row between two layers of filter papers wetted with deionized water. Filter papers were rolled

inside a piece of waxed paper and set vertically in a beaker containing 1 inch (2.5 cm) of deionized water. The beaker was covered with plastic wrap to prevent evaporation and placed at 25°C in the dark. Germination was scored 5 days after seeding. *MRP2* double homozygous seeds had the same germination frequency as WT segregants and the WT inbred G00010BC. To test germination under cold stress, the seeds rolled up in the filter papers were incubated at 4 °C for 7 days in the dark, or at 8 °C for 7 days, or 4 °C for 5 days followed by 25 °C for 2 days. No significant difference in germination frequency was found between *MRP2* double homozygous knockouts and WT under any of the cold-stressed conditions.

[0146] The foregoing demonstrates that the elevated phosphate levels in MRP variants disclosed herein did not significantly alter seed germination.

[0147] Example 4: Phenotype of *MRP* gene knockouts in hybrid plants.

[0148] Gene-edited MRP variants also were tested in hybrid *Brassica* plants. Male inbred G00555MC has an MRP2 gene on chromosome A9 (MRP2.A9) and two MRP2 genes on chromosome C9 (MRP2.C9a and MRP2.C9b). Gene editing in G00555MC was performed using the gRNA BNA-MRP2-C2 (SEQ ID NO:16).

[0149] Transformation of CRISPR-Cas gene editing system was done as in Example 1. Regenerated plantlets were screened using a NGS sequencing assay to obtain the knockout variants identified herein. Table 4 shows various mutations generated in the *MRP2* gene variants using the indicated gRNA sequence. In Table 4, a period indicates the double-stranded break point that was targeted using gRNA BNA-MRP2-C2 (SEQ ID NO:16); underlined residues represent nucleotide insertions; dashes represent nucleotide deletions. The following variants were made in MRP2.C9a: MRP2.C9_v3, MRP2.C9_v4, MRP2.C9_v5, MRP2.C9_v6; and MRP2.C9_v7; and in MRP2.C9b: MRP2.C9_v3 and MRP2.C9_v4.

TABLE 4

Sequence Listing	Sequence	Name of variant / mutation
SEQ ID NO:44 positions 2082-2117	AGGCTTGGGAGGATCGG.TACAGGGTCGTGCTCGAGG	wild type MRP2.A9 / none (G00555MC)
SEQ ID NO:34 positions 2082-2118	AGGCTTGGGAGGATCGG**A**TACAGGGTCGTGCTCGAGG	MRP2.A9_v6 / Insertion
SEQ ID NO:35 positions 2082-2118	AGGCTTGGGAGGATCGG**C**TACAGGGTCGTGCTCGAGG	MRP2.A9_v7 / Insertion

SEQ ID NO:36 positions 2107-2143	AGGCTTGGGAGGATCGG**T**TACAGGGTCGTGCTCGAGG	MRP2.A9_v8 / Insertion
SEQ ID NO:45 position 2033-2068	AGGCTTGGGAGGATCGG.TACAGGGTCGTGCTTGAGG	wild type MRP2.C9a/ none (G00555MC)
SEQ ID NO:37 position 2033-2069	AGGCTTGGGAGGATCGG**A**TACAGGGTCGTGCTTGAGG	MRP2.C9a_v3 / Insertion
SEQ ID NO:38 position 2033-2069	AGGCTTGGGAGGATCGG**T**TACAGGGTCGTGCTTGAGG	MRP2.C9a_v4 / Insertion
SEQ ID NO:39 position 2033-2069	AGGCTTGGGAGGATCGG**G**TACAGGGTCGTGCTTGAGG	MRP2.C9a_v5 / Insertion
SEQ ID NO:40 position 2033-2067	AGGCTTGGGAGGATCG–.TACAGGGTCGTGCTTGAGG	MRP2.C9a_v6 / Deletion
SEQ ID NO:41 position 2033-2065	AGGCTTGGGAGGAT––––ACAGGGTCGTGCTTGAGG	MRP2.C9a_v7 / Deletion
SEQ ID NO:46 Positions 1493-1528	AGGCTTGGGAGGATCGG.TACAGGGTCGTACTTGAGG	wild type MRP2.C9b / none (G00555MC)
SEQ ID NO:42 Positions 1493-1529	AGGCTTGGGAGGATCGG**A**TACAGGGTCGTACTTGAGG	MRP2.C9b_v8 / Insertion
SEQ ID NO:43 Positions 1493-1529	AGGCTTGGGAGGATCGG**T**TACAGGGTCGTACTTGAGG	MRP2.C9b_v9 / Insertion

[0150] Triple homozygous knockout plants (homozygous null for MRP2.A9, MRP2.C9a, and MRP2.C9b) were selected using a NGS sequencing assay as described in Example 1. Free or inorganic phosphate (Pi) content of the triple homozygous knockout was about 72.5 µmole/g of seed as compared to a wild type control that had Pi content of 22.3 µmole/g of seed. See Fig. 3.

[0151] The gene-edited female inbred G00010BC was converted to male sterile by carrying the pollen of G00010BC-MRP2 to the cytoplasmic male-sterile G00010FC, followed by two additional generations of crossing and selection for homozygous MRP2 knockouts. Hybrid F1 seeds were produced in growth chamber and F2 seeds were generated for seed composition analysis. The Pi content of the MRP2 hybrid was 65.1 µmole/g of seed as compared to wild type level of 6.8 µmole/g seed. See Fig. 4.

[0152] The foregoing provides examples of MRP variants in inbreds of different genetic backgrounds; and it also demonstrates that the inorganic phosphate trait seen in such inbreds is maintained in hybrids plants produced therefrom.

[0153] Example 5: Field testing of *MRP* gene knockouts.

[0154] Field testing was carried out to evaluate MRP2 knockouts for agronomic performance and seed Pi content. Inbreds and hybrids were grown in single-row plots using a randomized complete block design with three replications in field environments in Rockwood, ON, Canada. Fertilizer was applied to achieve maximum yields. Weeds and pests were controlled according to local practices. Number of emerged plants was counted 3 weeks after planting, plant vigor was rated on 1-9 scale (1=Low, 9=High) at 20 days after emergence, and flowering time was recorded as number of days after planting to the day when 50% of the plants in a plot have at least one flower in bloom. Seed samples were taken at maturity from plants in the middle of plots. Protein and oil contents were analyzed using a near infrared (NIR) spectrometer. To reduce pollinators carrying pollen around the field, plants were covered with white knitted shade cloth (20% density) during flowering time. Wind and physical contact with plants in neighboring rows can still cause cross pollination; and genotyping revealed that an approximately 10% of the seeds from homozygous plants were heterozygous in 2021.

[0155] Pi content of MRP2 double knockout inbred G00010BC was 45.2 μmole/g seed as compared to 9.2 μmole/g seed in WT control in 2021. See Fig. 5.

[0156] In 2022, the MRP2 knockouts had Pi contents of 34.9 μmole/g seed of G00010BC and 38.8 μmole/g seed of G00555MC, respectively. Wild type inbreds had Pi contents of 5.3 and 8.9 μmole/g seed. MRP2 hybrid F2 seeds had Pi content of 35.2 μmole/g seed; while the WT hybrid had 5.9 μmole/g seed. These results for 2022 field trials are shown in Fig. 6.

[0157] Seed oil and protein content were not affected. Additionally, no difference was observed in emergence count, plant vigor and flowering time between the MRP2 plants and wild-type controls.

[0158] A Pi increase was observed in homozygous MRP knockouts relative to WT controls under field conditions.

[0159] The foregoing provides examples of MRP variants in inbreds of different genetic backgrounds; and it demonstrates that the inorganic phosphate trait seen in such inbreds is maintained in hybrids plants produced therefrom. The foregoing also shows that the elevated inorganic phosphate trait provided by MRP variants did not affect the agronomic properties of hybrid plants.

[0160] Example 6: *MRP* knockout trait interactions with other grain traits.

[0161] *MRP*-conferred high seed Pi trait was tested for compatibility with other grain traits such as low fiber and low glucosinolates. *MRP2* knockout plants were crossed with a line having low-ADF (low acid detergent fiber) caused by *TT8* loss-of-function variants generated by gene editing. Homozygous *MRP2* plants, *TT8* plants, *MRP2/TT8* stacked plants, and wild type plants were identified in the F2 population. Seed composition analysis showed that the Pi content in *MRP2/TT8* stacks was similar to that in MRP2 plants and was higher than *TT8* and wild type. Presence of the *MRP2* gene-edited variants doesn't affect ADF reduction caused by *TT8* mutation.

[0162] In another experiment involving a *TT2*-mediated low ADF phenotype, *MRP2* and *TT2* genes were knocked out in the same plants, both the high-Pi and low-ADF phenotype was observed in *MRP2/TT2* plants. See Fig. 7 showing that seed phosphate levels in the *MRP2/TT2* double knockouts were much higher than wild type (WT) plants; see also Fig. 8 showing that ADF levels of *MRP2/TT2* were significantly reduced relative to WT plants.

[0163] The foregoing data demonstrate that the high-Pi trait of the disclosed *MRP* variants are compatible with low ADF traits and that these two traits can be combined in *Brassica* seed that is particularly suitable for use in animal meal, e.g., for monogastric animals.

[0164] Example 7: *MRP2* and *MRP1* Knockout Mutants generated by EMS mutagenesis

[0165] Ethyl methanesulfonate (EMS) mutagenesis population was developed. Dry seeds of canola inbred BC were treated with 0.3%, 0.5% and 0.8% of EMS, rinsed with distilled water, and the treated seeds (M1) were air-dried. M1 seeds were planted in the field and M2 seeds were harvested from 3,200 individual M1 plants. Three seeds from each M2 were planted out in greenhouse and 7,000 M2 plants were obtained. Leaf samples were taken from individual M2 plants for DNA extraction and M3 seeds were harvested. Of the 7,000 M2 plants, the genome of 550 lines were sequenced.

[0166] Multiple lines carrying MRP2 and MRP1 knockout mutation were identified by sequencing. MRP2 and MRP1 knockout mutants included nonsense mutation and splicing site mutations. Table 5 shows the name for each MRP2 and MRP1 knockout mutants obtained, and describes the mutation: indicating chromosome, nucleotide alteration and position within the indicated sequence listing, as well as the altered encoded amino acid alteration.

Table 5

Mutant Name	Mutant Description	Sequence Listing
MRP2.A9_m1	MRP2.A9 mutation G1871 to A, Trp419 to STOP	SEQ ID NO:53
MRP2.A9_m2	MRP2.A9 mutation G2168 to A, Trp518 to STOP	SEQ ID NO:54

MRP2.A9_m3	MRP2.A9 mutation C3370 to T, Gln820 to STOP	SEQ ID NO:55
MRP2.A9_m4	MRP2.A9 mutation C4022 to A, Ser1037 to STOP	SEQ ID NO:56
MRP2.A9_m5	MRP2.A9 mutation C5266 to T, Gln1322 to STOP	SEQ ID NO:57
MRP2.C9_m1	MRP2.C9 mutation A1381 to T, Lys279 to STOP	SEQ ID NO:58
MRP2.C9_m2	MRP2.C9 mutation G1445 to A, Trp300 to STOP	SEQ ID NO:59
MRP2.C9_m3	MRP2.C9 mutation C2377 to T, Gln611 to STOP	SEQ ID NO:60
MRP2.C9_m4	MRP2.C9 mutation G2439 to A, Trp631 to STOP	SEQ ID NO:61
MRP2.C9_m5	MRP2.C9 mutation C3586 to T, Gln917 to STOP	SEQ ID NO:62
MRP2.C9_m6	MRP2.C9 mutation C3610 to T, Arg925 to STOP	SEQ ID NO:63
MRP2.C9_m7	MRP2.C9 mutation G3726 to A, Trp963 to STOP	SEQ ID NO:64
MRP2.C9_m8	MRP2.C9 mutation C4166 to T, Gln1083 to STOP	SEQ ID NO:65
MRP2.C9_m9	MRP2.C9 mutation C4659 to T, Splice donor	SEQ ID NO:66
MRP2.C9_m10	MRP2.C9 mutation G4814 to A, Trp1217 to STOP	SEQ ID NO:67
MRP1.A10_m1	MRP1.A10 mutation G861 to A, Trp105 to STOP	SEQ ID NO:68
MRP1.A10_m2	MRP1.A10 mutation G975 to A, Trp143 to STOP	SEQ ID NO:69
MRP1.A10_m3	MRP1.A10 mutation C1943 to T, Gln466 to STOP	SEQ ID NO:70
MRP1.A10_m4	MRP1.A10 mutation C2288 to T, Gln581 to STOP	SEQ ID NO:71
MRP1.A10_m5	MRP1.A10 mutation C3747 to T, Gln987 to STOP	SEQ ID NO:72
MRP1.A10_m6	MRP1.A10 mutation G4698 to A, Trp1253 to STOP	SEQ ID NO:73
MRP1.A10_m7	MRP1.A10 mutation C5163 to T, Gln1381 to STOP	SEQ ID NO:74
MRP1.C5_m1	MRP1.C5 mutation C1882 to T, Gln467 to STOP	SEQ ID NO:75
MRP1.C5_m2	MRP1.C5 mutation C1963 to T, Gln494 to STOP	SEQ ID NO:76
MRP1.C5_m3	MRP1.C5 mutation G1971 to A, Trp496 to STOP	SEQ ID NO:77
MRP1.C5_m4	MRP1.C5 mutation G2072 to A, Trp530 to STOP	SEQ ID NO:78
MRP1.C5_m5	MRP1.C5 mutation C2615 to T, Gln686 to STOP	SEQ ID NO:79
MRP1.C5_m6	MRP1.C5 mutation G2626 to A, Trp689 to STOP	SEQ ID NO:80
MRP1.C5_m7	MRP1.C5 mutation G3732 to A, Trp964 to STOP	SEQ ID NO:81
MRP1.C5_m8	MRP1.C5 mutation G4625 to A, Trp1217 to STOP	SEQ ID NO:82
MRP1.C5_m9	MRP1.C5 mutation C4689 to T, Gln1239 to STOP	SEQ ID NO:83

[0167] To generate double knockout mutants, a homozygous MRP2.A9-m3 plant and MRP2.C9-m6 plant were crossed, F1 was self-pollinated, and F2 plants were genotyped. The MRP2 double knockout homozygous plants were identified.

Claims

1. A method of increasing inorganic phosphate in *Brassica napus* plant, seed, tissue or germplasm thereof, the method comprising introducing a targeted alteration to the sequence of one or more multidrug resistance-associated protein (*MRP*) genes *MRP1*, *MRP2*, or both *MRP1* and *MRP2* and thereby generating a *Brassica napus* plant, seed, tissue or germplasm thereof comprising one or more *MRP* variants and an increased level of inorganic phosphate relative to the plant, seed, tissue or germplasm thereof prior to introducing the one or more *MRP* variants.

2. The method of claim 1, comprising introducing a targeted alteration of one or more alleles of *MRP2* on chromosome A09 or C09 or introducing a targeted alteration of one or more alleles of *MRP1* on chromosome A10 or C05.

3. The method of claim 1 or 2, wherein the targeted alteration is an insertion or deletion that reduces or eliminates expression of the full-length protein encoded by the targeted *MRP* gene alleles.

4. The method of claim 3, wherein the targeted alteration introduces a premature termination codon in the one or more *MRP* variants.

5. The method of claim 1, wherein the targeted alteration introduces a premature termination codon in the position shown in Table 2 for *MRP2* on chromosome A09 or C09, Table 3 for *MRP1* on chromosome A10 or C05, or Table 4 for *MRP2* on chromosome A09 or C09.

6. The method of claim 1, wherein each of the one or more *MRP* variants comprises a premature termination codon or splice donor shown in SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83.

7. The method of any one of claims 1-6, wherein the method comprises introducing double homozygous variants of *MRP1* or *MRP2* and thereby generating *Brassica napus* plant, seed, tissue or germplasm thereof comprising double homozygous variants of *MRP1* or *MRP2*.

8. A method of producing high inorganic phosphate *Brassica napus* meal, the method comprising

a. selecting *Brassica napus* seed or grain comprising one or more *MRP* variants, wherein the variants comprise a targeted alteration of *MRP1, MRP2,* or both *MRP1* and *MRP2* and the selected seed or grain comprise increased inorganic phosphate relative to control seed or grain lacking the one or more *MRP* variants;

b. milling the selected seed or grain to produce high inorganic phosphate *Brassica napus* meal.

9. The method of claim 8, wherein the selected seed or grain comprises a targeted alteration to one or more alleles of *MRP2* on chromosome A09 or C09 or a targeted alteration to one or more alleles of *MRP1* on chromosome A10 or C05.

10. The method of claim 9, wherein the selected seed or grain comprises an insertion or deletion that reduces or eliminates expression of the full-length protein encoded by the targeted *MRP* genes.

11. The method of claim 10, wherein the insertion or deletion introduces a premature termination codon in the one or more *MRP* variants.

12. The method of claim 11, wherein the targeted alteration introduces a premature termination codon in the position shown in Table 2 for *MRP2* on chromosome A09 or C09, Table 3 for *MRP1* on chromosome A10 or C05, or Table 4 for *MRP2* on chromosome A09 or C09.

13. The method of claim 8, wherein the one or more *MRP* variants comprise one or more of the premature stop codons or splice variant shown in SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ

ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83, including combinations thereof.

14. The method of any one of claims 8-13, wherein the selected seed or grain comprises double homozygous variants of *MRP1* or *MRP2*.

15. The method of any one of claims 8-14, wherein the selected seed or grain further comprises a targeted alteration of one or more of the following genes: *LPA1, TT2, TT8, DFR, F3H, ANR, LDOX, MYB28* or *MAM1*.

16. *Brassica napus* plant, seed, tissue or germplasm thereof comprising one or more multidrug resistance-associated protein (*MRP*) gene variants which are single homozygous *MRP2* variants on chromosome A09 or C09; double homozygous *MRP2* variants on chromosomes A09 and C09, single homozygous *MRP1* variants on chromosome A10 or C05; or double homozygous *MRP1* variants on chromosomes A10 and C05, wherein the *Brassica napus* plant, seed, tissue or germplasm thereof comprising the one or more *MRP* variants have an increased level of inorganic phosphate relative to a control lacking the one or more *MRP* variants.

17. The *Brassica napus* plant, seed, tissue or germplasm thereof of claim 16, which comprises a combination of (i) single homozygous *MRP2* variants on chromosome A09 or C09 and single homozygous *MRP1* variants on chromosome A10 or C05, (ii) double homozygous *MRP2* variants on chromosome A09 and C09 and single homozygous *MRP1* variants on chromosome A10 or C05; or (iii) single homozygous *MRP2* variants on chromosome A09 or C09 and double homozygous *MRP1* variants on chromosome A10 and C05.

18. The *Brassica napus* plant, seed, tissue or germplasm thereof of claim 16 or 17, wherein each of the *MRP* variants comprises a premature termination codon in the position shown in Table 2 for *MRP2* on chromosome A09 or C09, Table 3 for *MRP1* on chromosome A10 or C05, or Table 4 for *MRP2* on chromosome A09 or C09.

19. The *Brassica napus* plant, seed, tissue or germplasm thereof of any one of claims 16 or 17, wherein each of the one or more *MRP* variants comprises a premature stop codon or splice variant shown in SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38,

SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, or SEQ ID NO:43, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, or SEQ ID NO:83.

20. The *Brassica napus* plant, seed, tissue or germplasm thereof of any one of claims 16-19, further comprising a targeted alteration of one or more of the following genes: *LPA1, TT2, TT8, DFR, F3H, ANR, LDOX, MYB28* or *MAM1*.

21. A method of identifying a plant, seed, tissue or germplasm thereof comprising an *MRP* variant associated with increased inorganic phosphate level, the method comprising:

a. providing plant, seed, tissue or germplasm thereof comprising one or more *MRP* variants in accordance any one of claims 14-18;

b. obtaining a sample comprising nucleic acid from the plant, seed, tissue or germplasm thereof;

c. screening the sample for any of the following:

i. the one or more *MRP* variants;

ii. one or more marker alleles within 5 cM of the one or more *MRP* variants (i) and linked to and associated with (i); and

d. detecting the one or more (i) *MRP* variants or (ii) marker alleles in the sample and thereby identifying the plant as having an *MRP* variant associated with increased inorganic phosphate level.

22. A method of introducing an *MRP* variant into a *Brassica napus* plant comprising:

a. crossing a first parent *Brassica napus* plant with a second parent *Brassica napus* plant to produce progeny plants, wherein the first parent plant is a plant comprising one or more *MRP* variants in accordance with any one of claims 16-20; and

b. selecting at least one progeny plant comprising the one or more *MRP* variants.

23. The method of claim 22 further comprising:

c. crossing the selected progeny plants with the second parent plant to produce backcross progeny plants; and

d. selecting for backcross progeny plants comprising the one or more *MRP* variants.

24. A recombinant nucleic acid construct comprising one or more of SEQ ID NOs:13-43 or SEQ ID NOs:53-83.

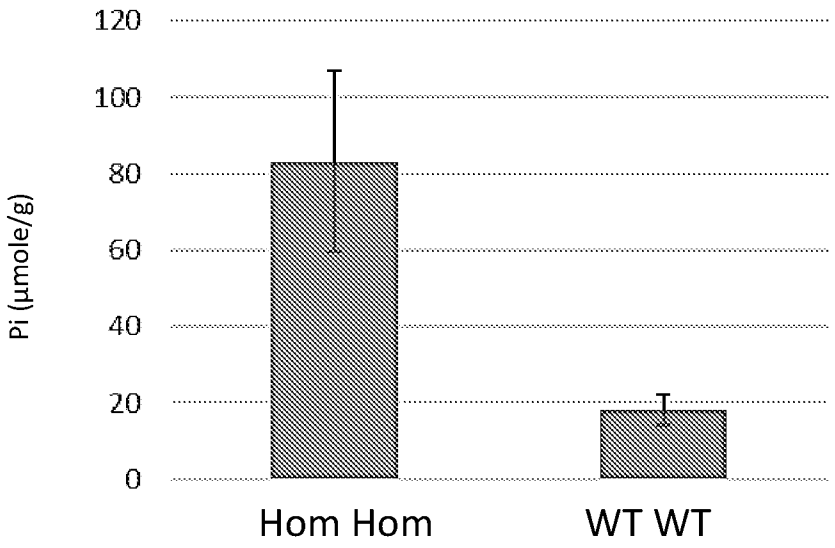
120
100
80
60
40
20
0
Pi (µmole/g)
Hom Hom
WT WT

FIG. 1

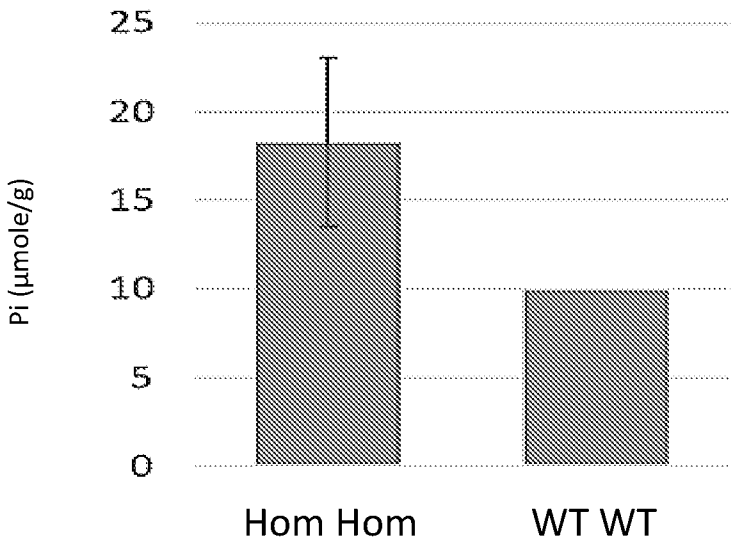
25
20
15
10
5
0
Pi (µmole/g)
Hom Hom
WT WT

FIG. 2

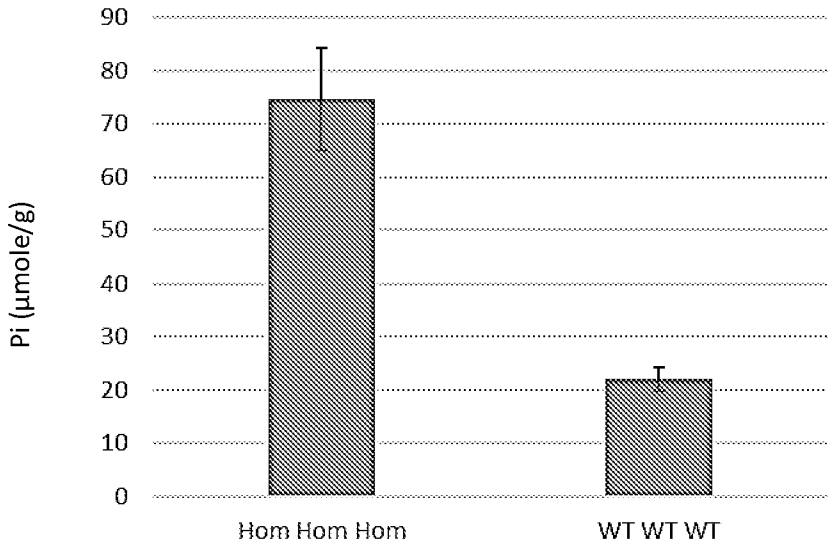
90
80
70
60
50
40
30
20
10
0
Pi (µmole/g)
Hom Hom Hom
WT WT WT

FIG. 3

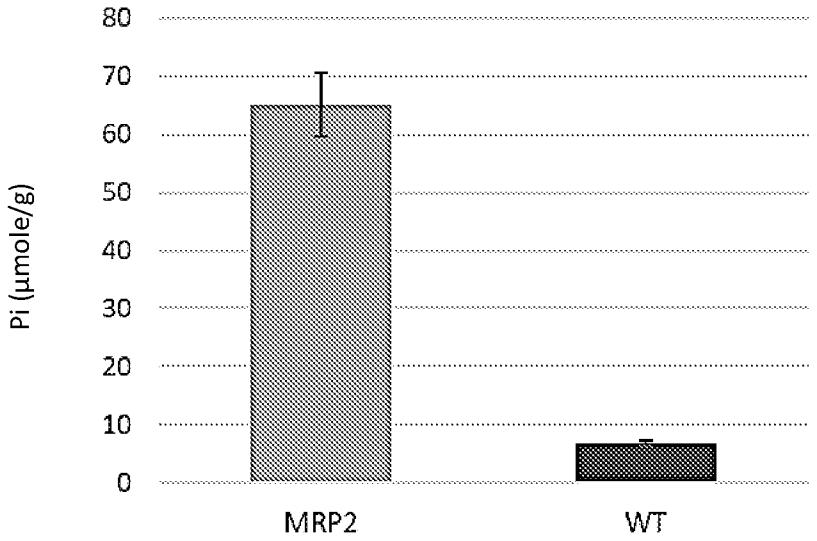
80
70
60
50
40
30
20
10
0
Pi (µmole/g)
MRP2
WT

FIG. 4

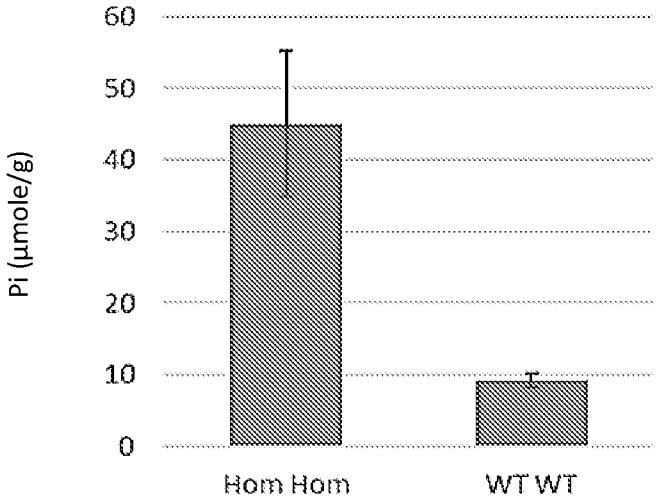
60
50
40
30
20
10
0
Pi (µmole/g)
Hom Hom
WT WT

FIG. 5

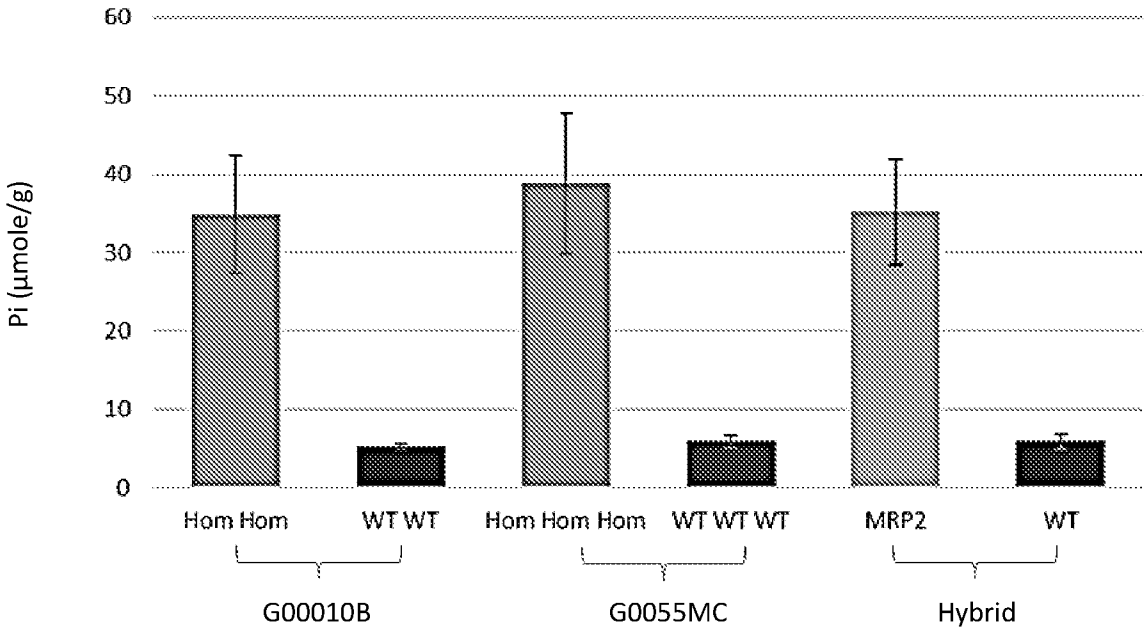
60
50
40
30
20
10
0
Pi (µmole/g)
Hom Hom
WT WT
Hom Hom Hom
WT WT WT
MRP2
WT
G00010B
G0055MC
Hybrid

FIG. 6

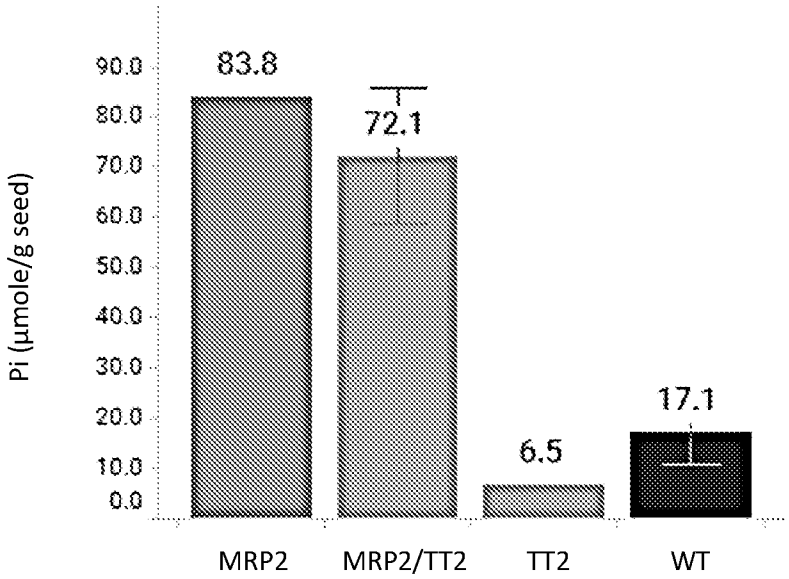
Pi (µmole/g seed)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
83.8
72.1
6.5
17.1
MRP2
MRP2/TT2
TT2
WT

FIG. 7

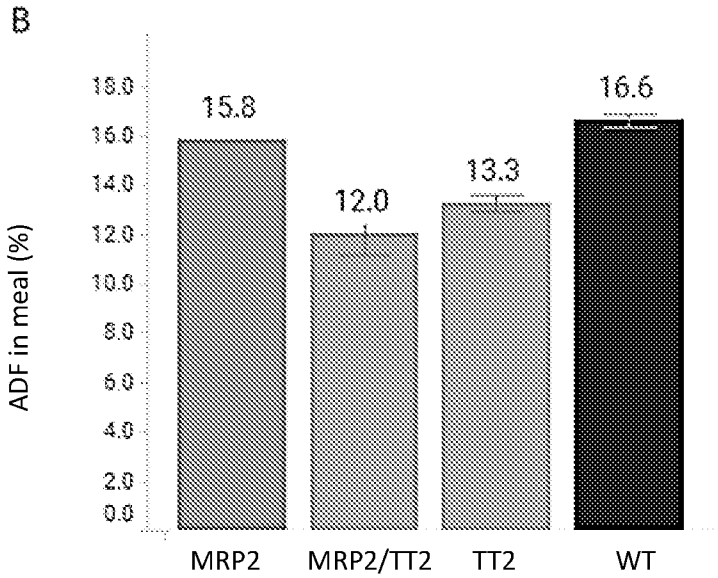
B
ADF in meal (%)
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
15.8
12.0
13.3
16.6
MRP2
MRP2/TT2
TT2
WT

FIG. 8