Mining of Potential Gene Resources for Breeding Nutritionally Improved Maize

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Abstract: Maize is one of the leading food crops and its kernel is rich in starch, lipids, protein and other energy substances. In addition, maize kernels also contain many trace elements that are potentially beneficial to human health, such as vitamins, minerals and other secondary metabolites. However, gene resources that could be applied for nutrient improvement are limited in maize. In this review, we summarized 107 genes that are associated with nutrient content from different plant species and identified 246 orthologs from the maize genome. In addition, we constructed physical maps and performed a detailed expression pattern analysis for the 246 maize potential gene resources. Combining expression profiles and their potential roles in maize nutrient improvement, genetic engineering by editing or ectopic expression of these genes in maize are expected to improve resistant starch, oil, essential amino acids, vitamins, iron, zinc and anthocyanin levels of maize grains. Thus, this review provides valuable gene resources for maize nutrient improvement.

Keywords: maize; nutrient improvement; homologous gene; biological engineering

1. Introduction

Maize (Zea mays L.) is one of the main food crops in the world, which stands first among the grain crops in terms of yield production. In addition to being used as food for humans, maize can also be used as animal feed or as raw material for industrial manufacturing. Maize kernels provide many nutrients, including starch, oil and protein, and are rich in microelements, such as vitamins and minerals. The pericarp has fiber and minerals; the aleurone layer contains high levels of minerals and antioxidants; the endosperm contains starch, protein, vitamins and antioxidants; and the embryo is rich in lipids, minerals and some vitamins [1]. Understanding the distribution of various nutrients facilitates the application of appropriate methods to obtain desired components (Figure 1).
Starch synthases (SSs) and starch branching enzymes (SBEs) catalyze the synthesis and degradation of starch, respectively. Starch synthases use glucose-1-phosphate (G-1-P) and ATP as substrates to synthesize starch molecules, while starch branching enzymes promote the formation of different starch structures.

Figure 1. Structure and nutrient distribution of maize kernels.

In face of the ever-changing demand for maize in the new era, traditional breeding strategies have the challenge of meeting human beings’ demands, from yield improvement to nutritional quality improvement. Compared with traditional breeding methods, molecular breeding significantly shortens the breeding process and has attracted more and more attention. Genome engineering technologies, including the CRISPR-Cas9 based genome editing and ectopic expression of functional genes driven by strong or tissue-specific promoters, have paved the way for molecular breeding [2,3]. So far, maize has the largest number of transgenic events that have been commercialized [4], which reflects the fact that maize improvement has attracted considerable attention and that genome engineering is profoundly changing the past and future of maize. Homologous genes among different plant species are highly likely to have similar functions. Many genes in model plant species such as rice (Oryza sativa) and Arabidopsis thaliana have been known to control specific traits. However, their homologs have not been identified or studied in maize. Therefore, delivering knowledge from model species to maize would be a rapid way to deliver maize nutrient improvement.

In this review, we focus on increasing the content of resistant starch, maize oil, essential amino acids, vitamins, minerals of iron and zinc, which are essential nutrients for human health. We summarize 107 genes that have been reported to be related to the above nutrient contents from different plant species, including A. thaliana, rice, soybean and potato, tomato, etc.; the protein sequences of these were used as queries to blast a maize genome with blastP on the Gramene website (http://ensembl.gramene.org/Zea_mays/Info/Index, accessed on 22 February 2022). All obtained sequences with low E-value (<10^-12) were selected for manual inspection. The Pfam domain searches (http://pfam.xfam.org/, accessed on 20 February 2022) were performed to confirm the candidate sequences as maize homologs. In addition, chromosomal mapping of these genes was carried out according to their positions on the chromosomes (Figure S1). Using available RNA_seq data, we analyzed the expression patterns of the 246 maize potential gene resources in maize early seeds, kernels and non-seed tissues. In addition, we also discuss the strategies of using these genes to obtain desired traits, providing a valuable candidate gene pool for nutrient improvement in maize.

2. Identification of Maize Potential Gene Resources for Starch Content Improvement

Starch accounts for most of the dry weight of corn kernels and provides calories for humans and animals. Starch comprises two types of polysaccharide molecules: amyllose (Am) and amylopectin (Ap). Am is a polysaccharide made of D-glucose units, almost all of which are linked by α-1,4-glycosidic bonds, while Ap molecules are linked by α-1,4-glycosidic bonds and α-1,6-glycosidic bonds [5]. Four major enzymes are involved in starch synthesis. ADP-glucose, a glucosyl donor for starch synthesis, is synthesized by the catalyzation of adenosine diphosphate glucose pyrophosphorylase (APGase), using glucose-1-phosphate (G-1-P) and ATP as substrates. Starch synthases (SSs) and starch branching enzymes (SBEs)
are responsible for elongating the glucose polymer and branching, respectively. Debranching enzymes (DBEs) catalyze the hydrolysis of α-1,6-branch linkages of starch and other branched polyglucans, and an isoamylase-type (ISA) debranching enzyme facilitates the crystallization of amylpectin by hydrolyzing some of the branches and thus is involved in amylpectin synthesis [6].

A type of starch, known as resistant starch, cannot be digested by the stomach and small intestine where it can be fermented by certain specialized microorganisms [7]. Resistant starch plays an essential role in human health, including lowering blood glucose and cholesterol levels [8]. The proportion of Am in starch was found to positively correlate with resistant starch content in sorghum [9]. Thus, improvement of Am content also indirectly increases the content of resistant starch.

Am content in maize kernels could be adjusted by altering the direction of starch synthesis. According to the starch synthesis process described above, SBE is the most critical factor in converting Am and Ap. Studies on rice [10,11], wheat [12], barley [13], potatoes [14,15] and cassava [16] showed the content of Am was increased when the activity of SBEs was suppressed, supporting the notion that manipulation of SBE is an effective way to increase the Am content. The effect of SSs on starch synthesis has been investigated and confirmed in rice [17,18] and sweet potatoes [19]. Granule bound starch synthase (GBSS) binds specially to starch and maintains the unbranching state of Am while the Protein Targeting to Starch 1 (PTST1) participates in the localization of GBSS in Am. Studies showed that boosting the expression of GBSS and PTST1 resulted in the enhancement of Am production [20–22]. Thus, we assume that harnessing these key enzymes involved in starch synthesis could also effectively improve the starch content in maize kernels. Sixteen homologous genes encoding these key enzymes were identified from the maize genome (Table 1).

### Table 1. List of potential gene resources for improving resistant starch content in maize.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|-------|-----------------|-----------------|---------|----------|------------|
| SBE   | starch branching enzyme | SBE1 Zm00001eb228530 | knockout | [10–16] |
|       |                  | SBE3 Zm00001eb357830 |         |          |            |
|       |                  | SBE4 Zm00001eb084160 |         |          |            |
|       |                  | AE1 Zm00001eb242610 |         |          |            |
| SS    | starch synthase  | SS1 Zm00001eb376100 | overexpression | [17–19] |
|       |                  | SS2 Zm00001eb070230 |         |          |            |
|       |                  | SS3 Zm00001eb431240 |         |          |            |
|       |                  | SS4 Zm00001eb353810 |         |          |            |
|       |                  | SS5 Zm00001eb191890 |         |          |            |
|       |                  | SS6 Zm00001eb222830 |         |          |            |
|       |                  | SS7 Zm00001eb194550 |         |          |            |
|       |                  | D1 Zm00001eb413290 |         |          |            |
|       |                  | SU2 Zm00001eb279740 |         |          |            |
| GBSS  | granule bound starch synthase | WX1 Zm00001eb378140 | overexpression | [20–22] |
|       |                  | GBSS1 Zm00001eb305810 |         |          |            |
| PTST1 | protein targeting to starch | GPM177 Zm00001eb231700 | overexpression | [20,21] |

### 3. Identification of Maize Potential Gene Resources for Oil Content Improvement

Corn oil is a byproduct of corn wet-milling industries, and is a significant part of the human diet, useful in industrial applications and an alternative to fossil fuels. Corn oil is mainly composed of 59% polyunsaturated (PUFA), 24% monounsaturated (MUFA) and 13% saturated fatty acid (SFA) [23]. To enhance the economic value of corn, genome engineering is efficient and effective in improving the oil content of corn kernels [24]. Many enzymes, carrier proteins and transcription factors (TF) associated with the regulation of oil yield have been identified in other species. Genes encoding these proteins are potential resources for generating high oil-yielding maize by genome engineering.
The chemical composition of oil is triacylglycerol (TAG) formed from the sequential acylation of three fatty acids (FAs), with glycerol-3-phosphate (G3P) as the backbone. TAG de novo synthesis is catalyzed in the Kennedy pathway and is affected by the glycolysis and tricarboxylic acid cycle (TCA) processes. Glyceraldehyde-3-phosphate dehydrogenase (GAPC) catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, and phosphoenolpyruvate carboxylase (PEPC) catalyzes the reaction of oxaloacetic acid to phosphoenolpyruvate. Overexpression of GAPC or silencing of PEPC promotes glycolysis and indirectly increases the content of dihydroxyacetone phosphate (DHAP) [25,26]. Glycerol-3-phosphate dehydrogenase (GPDH) converts DHAP into glycerol-3-phosphate (G3P). Acetyl-CoA, a product of TCA, is promoted to malonyl-CoA by acetyl-CoA carboxylase (ACC), which enters the Kennedy pathway together with G3P. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes G3P into lysocephatidic acid (LPA), which is the first step in glycerolipid biosynthesis [27]. LPA or phosphatidylincholine (PC) are further catalyzed by a series of enzymes, including diacylglycerol acyltransferase (DGAT) and phospholipid diacylglycerol acyltransferase (PDAT), and finally form TAG (Figure 2). Studies have shown that GPAT [28], DGAT [29–31] and PDAT [30] directly affect the TAG synthesis. Transcription factors of AtMYB89 [32], AtMYB96 [33], LEC [34–37], GL2 [38,39], FUS3 [40] and HB2 [41] are involved in TAG biosynthesis regulation.

Fatty acids are initially generated in the plastid and are transported to the endoplasmic reticulum for TAG synthesis. FAX1 and ABCA9 are identified as the carrier proteins for fatty acid transport from the plastid to the endoplasmic reticulum. In addition, Oleosin (OLE) encodes the most abundant seed oil droplet-specific protein, overexpression of which increases oil levels in rice and soybean [30,42]. On the other hand, silencing of sugar dependent 1 (SDP1), which inhibits the degradation of TAG, could also lead to increases in TAG content [31,43,44]. The TAG synthesis pathway and key enzymes are shown in Figure 2. Sixty-one homologous genes involved in TAG synthesis were identified from the maize genome (Table 2).
Table 2. List of potential gene resources for enhancing lipid yield in maize.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|-------|-----------------|-----------------|---------|----------|------------|
| GAPC  | glyceraldehyde-3-phosphate dehydrogenase | GPC1, GPC2, GPC3, GPC4 | Zm00001eb173410, Zm00001eb261430, Zm00001eb184000, Zm00001eb246370 | overexpression | [25] |
| PEPC2 | phosphoenolpyruvate carboxylase | PEP1 | Zm00001eb383680 | knockout | [26] |
| GPDH  | glycerol-3-phosphate dehydrogenase | GPDH1, GPDH2, GPDH3, GPDH4, GPDH5, GPDH6 | Zm00001eb141610, Zm00001eb369390, Zm00001eb352530, Zm00001eb139850, Zm00001eb303710, Zm00001eb419210 | overexpression | [45] |
| ACC1  | acetyl-CoA carboxylase | ACC1, ACC2, TIDP3607 | Zm00001eb419400, Zm00001eb6086560, Zm00001eb223980, Zm00001eb628820, Zm00001eb189990, Zm00001eb367400 | overexpression | [46,47] |
| GPAT9 | glycerol-3-phosphate acyltransferase | GPAT14, GPAT19 | Zm00001eb6396350, Zm00001eb323170 | overexpression | [28] |
| DGAT1 | diacylglycerol acyltransferase | LN1, DGAT2 | Zm00001eb277490, Zm00001eb284200 | overexpression | [29–31] |
| PDAT  | phospholipid diacylglycerol acyltransferase | PZA01735, TIDP3675 | Zm00001eb100310, Zm00001eb314300, Zm00001eb118700, Zm00001eb148010, Zm00001eb331670, Zm00001eb342120 | overexpression | [30] |
| MYB89 | transcription factor | MYB136 | Zm00001eb128770 | knockout | [32] |
| MYB96 | transcription factor | FDL1, MYB33, MYB35, MYB52, MYB70, MYB83, MYB162 | Zm00001eb328280, Zm00001eb6041330, Zm00001eb099570, Zm00001eb392230, Zm00001eb109860, Zm00001eb041320, Zm00001eb312600 | overexpression | [33] |
| LEC1  | transcription factor | LEC1 | Zm00001eb253260 | overexpression | [34,35] |
| LEC2  | transcription factor | ABI19 | Zm00001eb361390 | overexpression | [36,37] |
| GL2   | transcription factor | OCL1 | Zm00001eb126140 | knockout | [38,39] |
| FUS3  | transcription factor | ABI19 | Zm00001eb361390 | overexpression | [40] |
| HB2   | transcription factor | HB2 | Zm00001eb293010 | overexpression | [41] |
| FAX1  | carrier protein | FAX1, ZIM25 | Zm00001eb301150, Zm00001eb379540, Zm00001eb317650, Zm00001eb424650 | overexpression | [48] |
| ABCA9 | carrier protein | | Zm00001eb042110 | overexpression | [49] |
| OLE   | delta-9 desaturase | OLE1, OLE3, OLE4 | Zm00001eb074940, Zm00001eb216880, Zm00001eb053890 | overexpression | [30,42] |
Table 2. Cont.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|-------|-----------------|-----------------|---------|----------|------------|
| SDP1  | sugar dependent | TGL1 AY110479   | Zm00001eb370460 Zm00001eb062080 | knockout | [31,43,44] |
| FAD2  | delta-12 fatty acid desaturase | FAD2 | Zm00001eb188990 Zm00001eb252730 Zm00001eb300860 Zm00001eb409700 Zm00001eb442020 | knockout | [50,51] |
| FAD3  | delta-12 fatty acid desaturase | FAD7 FAD8 | Zm00001eb397050 Zm00001eb013340 Zm00001eb163200 Zm00001eb111980 | knockout | [50] |
| FAE1  | fatty acid elongase | KCS1 KCS16 | Zm00001eb344070 Zm00001eb296230 | knockout | [51] |

4. Identification of Maize Potential Gene Resources for Essential Amino Acid Content Improvement

Essential amino acids are vital for protein synthesis, tissue repair and nutrient absorption. For instance, both lysine and tryptophan are important components of neurotransmitters. However, humans and animals cannot synthesize essential amino acids and can only get them from diets rich in proteins. Grains are low in lysine, while beans are poor in methionine. In maize kernels, protein content ranges from 7% to 14%, depending on genotype and environmental effects [1]. Here, we focus on improving the content of three important essential amino acids: methionine, lysine and tryptophan, as well as total protein (Figure 3).

![Figure 3. Schematic representation of amino acid biosynthetic pathways in plants. Curved arrows with a (−) sign represent major feedback inhibition loops by the end product amino acids and arrows with a (+) sign represent activation. (AK, aspartate kinase; TS, threonine synthase; CGS, cystathionine γ-synthase; HMT, homocysteine S-methyltransferase).](image)

Both lysine and methionine are synthesized from aspartate via different pathways. Aspartate kinase (AK) catalyzes the first step, which is a rate-limiting step that requires ATP, and this step is also regulated by subsequent steps in a feedback manner. Single amino acid substitution mutants of ak are insensitive to the feedback inhibition of lysine synthesis and elevate lysine content [52]. Asparaginyl-tRNA synthetase (SYNC) mediates the process of linking amino acids to tRNAs, and over-accumulation of the SYNC has been shown to increase lysine levels [53]. Increasing the abundance of proteins rich in lysine, such as VSP and BiP, elevates lysine content [54–56]. Cystathionine γ-synthase (CGS), the first specific enzyme for methionine synthesis using cysteine as a precursor, is critical for the control of methionine content [57,58]. The backflow from S-methylmethionine to methionine is mainly catalyzed by homocysteine S-methyltransferase (HMT). Threonine synthase (TS)
is a key enzyme for threonine synthesis but affects methionine formation as it competes with TS for their common substrate O-phosphohomoserine. More TS makes the resource flow to threonine, which is not conducive to the formation of methionine [59]. Studies have shown that mutations in ts boost the methionine level [60,61]. In the pathway of tryptophan synthesis, anthranilate synthase (ASA) is a key enzyme of the process and affects the pathway [62]. Aspartate aminotransferase (AAT) is also one of the important targets for improving protein content as it participates in the regulation of carbon and nitrogen metabolism through the transfer of the amino group [63]. Asparagine synthetase (ASN) has a similar function to AAT. Carrier protein SUT1 [64], AAP6 [65] and TF TaNAC100 [66] were also found to affect the protein content of grains. Twenty-eight homologous genes involved in essential amino acid biosynthesis were identified from the maize genome (Table 3).

Table 3. List of potential gene resources for elevating essential amino acid content in maize.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|-------|-----------------|----------------|--------|----------|------------|
| AK    | aspartate kinase| ASK1 ASK2     | Zm00001eb064530 Zm00001eb094670 | knockout | [52]       |
| SYN1  | asparaginyl-tRNA synthetase | Zm00001eb341390 |        | overexpression | [53]       |
| VSP   | storage protein | VSP1 VSP2     | Zm00001eb283450 Zm00001eb283460 | overexpression | [54,55]   |
| GRP   | storage protein | GRP1 GRP2     | Zm00001eb229930 Zm00001eb229940 | overexpression | [56]       |
| CGS   | cystathionine γ-synthase | CGS1 | Zm00001eb392050 Zm00001eb018300 | overexpression | [57,58]   |
| TSI   | threonine synthase | THR1 THR2 THR3 | Zm00001eb294790 Zm00001eb295790 Zm00001eb295890 | knockout | [60,61]    |
| HMT   | homocysteine S-methyltransferase | HMT1 | Zm00001eb399940 | overexpression | [67]       |
| ASA   | anthranilate synthase | ASA1 ASA2 | Zm00001eb063220 Zm00001eb211420 | overexpression | [68,69]   |
| AAT   | aspartate aminotransferase | GOT1 GOT2 GOT3 GOT4 | Zm00001eb152450 Zm00001eb257910 Zm00001eb258900 Zm00001eb146400 | overexpression | [63] |
| ASN1  | asparagine synthetase | ASN3 ASN4 | Zm00001eb013430 Zm00001eb396990 | overexpression | [70] |
| SUT1  | carrier protein | SUT1 SUT2 SUT3 SUT4 | Zm00001eb005460 Zm00001eb402200 | overexpression | [64] |
| AAP6  | carrier protein | AAP6 AAP7 | Zm00001eb145670 Zm00001eb145680 | overexpression | [65] |
| NAC100| transcription factor | NAC100 | Zm00001eb080700 | knockout | [66] |

5. Identification of Maize Potential Gene Resources for Vitamin Content Improvement

After decades of relentless efforts by plant breeders, the yield of staple crops has increased dozens of times. However, hidden hunger, which refers to eating food that is insufficient in vitamins and micronutrients, becomes a new problem that affects more than 2 billion people globally. Crops such as corn are unable to provide sufficient micronutrients and need to undergo biofortification, which uses agricultural methodologies to augment
the nutritional quality of food and counter micronutrient malnutrition [71]. Vitamins are essential micronutrients for growth, metabolism, reproduction and other processes related to human health. Vitamins can be classified into two groups: fat-soluble (A, D, E and K) and water-soluble (B and C).

β-carotene is a kind of red-orange pigment, which imparts color and antioxidant properties to plants and fruits. When it enters the body, it turns into vitamin A. Deficiency in vitamin A can cause night blindness. Isoprenoids produced by the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway are carotenoid precursors [72]. 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) are important catalytic enzymes for β-carotene synthesis. Phytoene desaturase (PDS) also promotes the formation of carotene. In addition to improving the synthesis efficiency through overexpressing key enzymes, some other factors that are not directly involved in the biosynthetic pathway have been shown to affect carotenoid accumulation in several plant species. Ectopic expression of ORANGE (OR), a plastidial DnaJ cysteine-rich domain-containing protein governing chromoplast biogenesis and carotenoid accumulation, promotes carotenoid accumulation and fruit development in tomatoes [73,74]. Suppression of de-etiolated 1 (det1) that affects plant light absorption via RNAi alters the carotenoid content in tomatoes and brassica napus [75,76]. Ectopic expression of a brassinazole-resistant 1 (bzr1–1d) transcription factor in brassinosteroid signaling enhances carotenoid accumulation in tomatoes [77]. Suppression of de-etiolated 1 (det1) that affects plant light absorption via RNAi alters the carotenoid content in tomatoes and brassica napus [75,76]. Ectopic expression of a brassinazole-resistant 1 (bzr1–1d) transcription factor in brassinosteroid signaling enhances carotenoid accumulation in tomatoes [77]. Transcription factors cytosine-mismatch-binding protein 1 (CMB1) and stay-green protein (SGR1) were found to regulate carotenoid accumulation during fruit ripening in tomatoes [78,79]. As an antioxidant, β-carotene is easily degraded by light, heat and oxygen. The inhibition of carotenoid cleavage dioxygenase (CCD) and lipoxygenase (LOX) can delay the degradation of β-carotene [80–82].

For mammals, deficiency in vitamin E is associated with some cancers, as well as neurodegenerative and cardiovascular diseases. Vitamin E is made up of four tocopherols and four tocotrienols, of which α-tocopherol is the most active form. The phytol moiety of tocopherols could be derived from chlorophyll. Chlorophyll dephytylase (CLD) and chlorophyll synthase (CHLG) are involved in the synthesis and decomposition of the process, respectively. e-hydroxyphenylpyruvate dioxygenase (HPPD), homogentisate phytyltransferase (HPT), homogentisic acid geranygeranyl transferase (HGGT), 2-methyl-6-phytylbenzoquinol methyltransferase (MPBQMT) and tocopherol cyclase (TC) participate in the next catalytic steps for tocopherol formation. Overexpression of these enzyme encoding genes is conducive to the synthesis of tocopherols. Studies have found that tocopherol-binding protein (TBP) is a transporter of tocopherols and that silencing of TBP reduces the content of tocopherols [83].

Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin and plays important roles in supporting cardiovascular function, immune cell development, iron utilization and other functions. Although ascorbic acid is an important antioxidant, it cannot be synthesized by humans and must be obtained from food. Many methods have been developed to increase the amount of ascorbic acid, and some of them have already been applied to maize. The synthetic reaction of ascorbic acid originates from glucose-6-phosphate (G6P); G6P is transformed into GDP-mannose through a series of enzymatic reactions, and then by the catalyzation of GDP-mannose 3,5-epimerase (GME), GDP-galactose phosphorylase (GGP), galactose-1-phosphate phosphatase (GPP), galactose dehydrogenase (GDH) and galactono-1,4-lactone dehydrogenase (GalLDH), finally forming ascorbic acid. All of these enzymes have been shown to contribute to the synthesis of tocopherols. Overexpression of these enzyme encoding genes is conducive to the synthesis of tocopherols. Animals and plants synthesize ascorbic acid through completely different pathways but both use L-gulono-1,4-lactone oxidase (GulLO). Therefore, GulLO is a common enzyme that can boost the ascorbic acid content both in animals and plants [84]. Besides, dehydroascorbate reductase (DHAR) can facilitate ascorbic acid regeneration [85,86].

Group B vitamins are a set of enzyme cofactors, and their derivatives include thiamin, riboflavin, niacin, pantothenate, pyridoxine, biotin, folate, cobalamin and so on. Group B vitamins also play a critical role in human health, coordinating the metabolism of the body,
but their mechanisms are not well understood. In the case of folate, GTP is its synthetic substrate, which is first catalyzed by GTP cyclohydrolase I (GTPCHI). After entering the mitochondrion, it merges with para-aminobenzoate (p-ABA) from plastid and is catalyzed by aminodeoxychorismate synthase (ADCS) and other enzymes. Overexpression of two vital enzymes, dihydrofolate synthetase (DHFS) or folylpolyglutamate synthase (FPGS), improves the efficiency of folate synthesis [87]. These enzymes add glutamate to folate and increase its stability, while γ-glutamyl hydrolase (GGH) hydrolyzes it. On the contrary, overexpression of GGH decreases the level of folate [88]. The key enzymes involved in vitamin A, B, C and E synthesis are shown in Figure 4. Fifty-six homologous genes involved in vitamin synthesis were identified from the maize genome (Table 4).

Figure 4. Schematic representation of vitamin biosynthetic pathways in plants. (G3P, glycerol-3-phosphate; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXP, 1-deoxy-D-xylulose-5-phosphate; DXR, 1-Deoxy-D-xylulose 5-phosphate reductoisomerase; IPP, isopentenyl diphosphate isomerase; PDS, phytoene desaturase; HPPD, ϱ-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate phytlytransferase; MPBQ, methylphytylbenzoquinol; HGGT, homogentisic acid geranylgeranyl transferase; TC, tocopherol cyclase; MPBQMT, 2-methyl-6-phytylbenzoquinol methyltransferase; DMPBQ, dimethylphytylbenzoquinone; PEP, phosphoenolpyruvate; E4P, erythrose 4-phosphate; ADCS, aminodeoxychorismate synthase; p-ABA, para-aminobenzoate; DHF, dihydrofolate; GTP, GTP cyclohydrolase I; DHFS, dihydrofolate synthetase; DHF, dihydrofolate; THF, tetrahydrofolate; FPGS, folylpolyglutamate synthase; G6P, glucose-6-phosphate; GME, GDP-mannose 3,5-epimerase; GGP, GDP-L-galactose phosphorylase; GPP, L-galactose-1-phosphate; GDH, L-galactono-1,4-lactone dehydrogenase; G6P, glucose-6-phosphate; GME, GDP-mannose 3,5-epimerase; GGP, GDP-L-galactose phosphorylase; GPP, L-galactose-1-phosphate; GDH, L-galactono-1,4-lactone dehydrogenase.)

Table 4. List of potential gene resources for enhancing vitamin contents in maize.

| Genes   | Protein Function                  | Maize Orthologs | Gene ID                        | Strategy   | References |
|---------|-----------------------------------|-----------------|-------------------------------|------------|------------|
| DXS     | 1-deoxyxylulose 5-phosphate synthase | DXS1            | Zm00001eb287860              | overexpression | [89]       |
| DXR     | 1-deoxy-D-xylulose 5-phosphate reductoisomerase | DXR1, DXR2     | Zm00001eb126690, Zm00001eb334370 | overexpression | [72]       |
| PDS     | phytoene desaturase                | VPS             | Zm00001eb006300              | overexpression | [90]       |
| OR      | coactivator                        |                 | Zm00001eb249060              | overexpression | [73,74]   |
| DET1    | transcription factor               |                 | Zm00001eb317230, Zm00001eb341540 | knockout    | [75,76]   |
| BZR1    | transcription factor               | BES1            | Zm00001eb325550              | overexpression | [77]       |
Table 4. Cont.

| Genes   | Protein Function            | Maize Orthologs | Gene ID                                                                 | Strategy          | References  |
|---------|----------------------------|-----------------|------------------------------------------------------------------------|-------------------|-------------|
| CMB1    | transcription factor        | ZMM6 ZMM7 ZMM27 | Zm00001eb036590 Zm00001eb317770 Zm00001eb102450                      | overexpression    | [78]        |
| SGR1    | magnesium dechelatase       | NYE1 NYE2       | Zm00001eb319560 Zm00001eb103480                                      | knockout          | [79]        |
| CCD4    | carotenoid cleavage dioxygenase | NCED6 NCED8  | Zm00001eb188280 Zm00001eb251990                                      | knockout          | [80,81]     |
| LOX1    | lipoxygenase                | LOX4 LOX5       | Zm00001eb054050 Zm00001eb216870                                      | knockout          | [82]        |
| CLD1    | chlorophyll dephytylase     | LMC2173         | Zm00001eb349130                                                      | overexpression    | [91]        |
| CHLG    | chlorophyll synthase        | CHLG1           | Zm00001eb286140                                                      | knockout          | [92]        |
| GPPD    | e-hydroxyphenylpyruvate dioxygenase | HPPD1 | Zm00001eb232960 Zm00001eb304950                                      | overexpression    | [93]        |
| HPT     | homogentisate phytlytransferase | HPT1           | Zm00001eb389370                                                      | overexpression    | [94]        |
| HGTT    | homogentisic acid geranylgeranyl transferase | HGTT1 HGTT2 HGTT3 | Zm00001eb386720 Zm00001eb105110 Zm00001eb121230 Zm00001eb382300 | overexpression    | [89]        |
| MPBQMT  | 2-methyl-6-phytylbenzoquinol methyltransferase | APG1 | Zm00001eb031790                                                      | overexpression    | [93]        |
| TC      | tocopherol cyclase          | SXD1            | Zm00001eb237270                                                      | overexpression    | [94]        |
| TBP     | tocopherol-binding protein  |                 | Zm00001eb197980 Zm00001eb347610                                      | overexpression    | [83]        |
| GULLO   | L-gulono-1,4-lactone oxidase |                 | Zm00001eb059530 Zm00001eb072160 Zm00001eb154880 Zm00001eb236290 Zm00001eb421440 Zm00001eb236880 | overexpression    | [95]        |
| GME     | GDP-mannose 3,5-epimerase   | GME1 GME2       | Zm00001eb047980 Zm00001eb167750                                      | overexpression    | [96]        |
| GGP     | GDP-L-galactose phosphorylase | SI946084H12   | Zm00001eb144410                                                      | overexpression    | [96]        |
| GPP     | L-galactose-1-phosphate phosphatase | GPP1 | Zm00001eb049310                                                      | overexpression    | [96]        |
| GDH     | L-galactose dehydrogenase   | GALDH1          | Zm00001eb408730                                                      | overexpression    | [96]        |
| GALLDH  | L-galactono-1,4-lactone dehydrogenase | GLDH1 | Zm00001eb093120                                                      | overexpression    | [96]        |
| DHAR1   | dehydroascorbate reductase  | DHAR1 DHAR2 DHAR3 | Zm00001eb355540 Zm00001eb355550 Zm00001eb266260 | overexpression    | [85,86]     |
| GTPCHI  | GTP cyclohydrolase          | GCH1 GCH2       | Zm00001eb067370 Zm00001eb432940                                      | overexpression    | [87]        |
| ADCS    | aminodeoxychorismate synthase | ADCS1          | Zm00001eb272970                                                      | overexpression    | [87]        |
| DHFS    | dihydrofolate synthetase    | DHFS1 DHFS2     | Zm00001eb410070 Zm00001eb137120                                      | overexpression    | [87]        |
Table 4. Cont.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|--------|------------------|-----------------|---------|----------|------------|
| FPGS   | folicpolyglutamate synthase | FGP2 BM4 | Zm00001eb044170 Zm00001eb404110 Zm00001eb299330 Zm00001eb421680 | overexpression | [87] |
| GGH    | γ-glutamyl hydrolase | | Zm00001eb199250 Zm00001eb353180 | overexpression | [88] |

6. Identification of Maize Potential Gene Resources for Mineral Content Improvement

Minerals can be used as the components of some special substances in the human body and also as a co-enzyme to participate in metabolism as well as maintain cell membrane permeability and other various functions. It is important to understand mineral transport processes, since the minerals within food need to be taken up by plants from the soil. Plants have evolved two strategies for iron absorption. In dicots, Fe$^{3+}$ is reduced to Fe$^{2+}$ and then transported into cells. Unlike this, grass plants, such as maize, could directly chelate Fe$^{3+}$ by mugineic acid (MA) for transport. However, rice uses both strategies for iron uptake [97]. Overexpression of nicotianamine synthase (NAS) and nicotianamine amino-transferase (NAAT), both of which participate in MA biosynthesis, facilitates the transport of iron [98,99]. NRAMP1 and NRAMP5 are two important transporters responsible for transporting Fe from roots to above-ground tissues where Fe could be stored in seeds. Fe chelates with citrate as it flows through the vascular, and FRD3 and FRDL1 are involved in the citrate transport. Fe is stored in vacuoles in plants, and VIT1 and NRAMP4 are responsible for the positive and negative regulation of vacuolar Fe content, respectively. Vacular Fe stores can be used to increase endosperm Fe content by inhibiting or promoting the expression of VIT1 and NRAMP4, respectively [100,101]. Overexpression of endosperm Fe storage protein FER significantly increases Fe content in the endosperm [102]. Many TFs have been found to regulate Fe uptake and transport from different plant species, including IRO2 [103], OsbHLH58 [104], OsbHLH59 [104], AtbHLH29 [105], GmbHLH300 [106], IDEF1 [107] and CSN6 complex [108]. In addition, Rab6a, as a subunit of small GTPase, is involved in adaption to CO$_2$ enrichment, thereby regulating photosynthesis and Fe content [109]. Fe-binding ubiquitin ligase (HRZ) is associated with the negative regulation of the Fe transport pathway [110]. Zn transport is similar to Fe, and overexpression of NAS and NAAT also increases Zn content. However, many transporters are unique for Zn transport; these include MTP1 [111], ZIF1 [112], ZIF2 [113], HMA2 [114], HMA4 [115], HMA7 [116], ZIP1 [117], ZIP8 [118] and so on.

Despite the fact that improving the transport efficiency of microelements could increase their contents in plants, plants contain a special anti-nutrient myo-inositol 1,2,3,4,5,6-hexakisphosphate (InsP6), commonly known as phytic acid (PA), which seriously affects human absorption of minerals. PA strongly chelates cations to form phytate, an insoluble salt that blocks the absorption of Fe and Zn from the human gut. The first step in the PA synthesis pathway is the conversion of glucose-6-phosphate to myo-inositol-3-phosphate by myo-inositol-1-phosphate synthase (MIPS), following which the myo-inositol-3-phosphate is further phosphorylated by 2-phosphoglycerate kinase (PGK), inositol 1,3,4-trisphosphate 5/6-kinase (ITPK) and inositol 1,3,4,5,6-pentakisphosphate 2-kinase (IPK), to finally form PA [119]. PA could be downregulated by either inhibiting the production of these enzymes or promoting the synthesis of phytases such as HAD and PAP. Seventy-six homologous genes involved in mineral absorption, transport and regulation were identified from the maize genome (Table 5).
Table 5. List of potential gene resources for enhancing mineral contents in maize.

| Genes | Protein Function          | Maize Orthologs | Gene ID            | Strategy   | References |
|-------|---------------------------|-----------------|--------------------|------------|------------|
| NAS   | nicotianamine synthase    |                 | NAS1 Zm00001eb396230 | overexpression | [98]       |
|       |                           |                 | NAS2 Zm00001eb014700 |            |            |
|       |                           |                 | NAS3 Zm00001eb052890 |            |            |
|       |                           |                 | NAS4 Zm00001eb218440 |            |            |
|       |                           |                 | NAS6 Zm00001eb36110  |            |            |
|       |                           |                 | NAS8 Zm00001eb36250  |            |            |
|       |                           |                 | NAS9 Zm00001eb014680 |            |            |
|       |                           |                 | NAS10 Zm00001eb36280  |            |            |
| NAAT  | nicotianamine aminotransferase |               | NAAT1 Zm00001eb203230 | overexpression | [99]       |
|       |                           |                 | PCO115235C Zm00001eb240650 |            |            |
| NRAMP1| carrier protein           |                 | NRAT1 Zm00001eb224770 | overexpression | [120]      |
| NRAMP5| carrier protein           |                 | NRAT5 Zm00001eb304610 | overexpression | [121]      |
| FRD3  | carrier protein           |                 | MATE1 Zm00001eb261140 | overexpression | [122]      |
|       |                           |                 | MATE1 Zm00001eb143800 |            |            |
|       |                           |                 | MATE1 Zm00001eb424530 |            |            |
| FRDL1 | carrier protein           |                 | MATE3 Zm00001eb008790 | overexpression | [123]      |
| VIT1  | carrier protein           |                 | Zm00001eb424350      | knock-out  | [100]      |
|       |                           |                 | Zm00001eb099160      |            |            |
|       |                           |                 | Zm00001eb312010      |            |            |
| NRAMP3| carrier protein           |                 | NRAT3 Zm00001eb400560 | overexpression | [101]      |
|       |                           |                 | NRAT4 Zm00001eb030500 |            |            |
|       |                           |                 | NRAT4 Zm00001eb051790 |            |            |
| FER   | storage protein           |                 | FER1 Zm00001eb195010 | overexpression | [102]      |
|       |                           |                 | FER2 Zm00001eb404870 |            |            |
| IRO2  | transcription factor      |                 | BHLH54 Zm00001eb362800 | overexpression | [103]      |
|       |                           |                 | BHLH126 Zm00001eb140680 |            |            |
| BHLH58| transcription factor      |                 | BHLH118 Zm00001eb289490 | overexpression | [104]      |
| BHLH59| transcription factor      |                 | BHLH128 Zm00001eb209480 | overexpression | [104]      |
|       |                           |                 | BHLH129 Zm00001eb229950 |            |            |
| BHLH29| transcription factor      |                 | BHLH100 Zm00001eb420910 | overexpression | [105]      |
|       |                           |                 | BHLH101 Zm00001eb085690 |            |            |
| BHLH300| transcription factor      |                 | BHLH54 Zm00001eb362800 | overexpression | [106]      |
| IDEF1 | transcription factor      |                 | ABI47 Zm00001eb198710 | overexpression | [107]      |
|       |                           |                 | ABI49 Zm00001eb259870 |            |            |
| CSN6  | coactivator               |                 | Sl605023C06B Zm00001eb199540 | knock-out | [108]      |
|       |                           |                 | Zm00001eb034040      |            |            |
| RAB6A | small GTPase              |                 | IDP871 Zm00001eb006940 | overexpression | [109]      |
| HRZ   | Fe-binding ubiquitin ligase |               | 541975 Zm00001eb360580 | knock-out  | [110]      |
|       |                           |                 | Zm00001eb156300      |            |            |
|       |                           |                 | Zm00001eb294920      |            |            |
| MTP1  | carrier protein           |                 | UMC2311 Zm00001eb265000 | overexpression | [111]      |
|       |                           |                 | Zm00001eb385520      |            |            |
|       |                           |                 | Zm00001eb420140      |            |            |
|       |                           |                 | Zm00001eb354910      |            |            |
Table 5. Cont.

| Genes | Protein Function | Maize Orthologs | Gene ID | Strategy | References |
|-------|-----------------|-----------------|---------|----------|------------|
| ZIF1  | carrier protein | MFSD1 MFSD2 IDP8516 TOM3 UMC1028 IDP7324 YS3 IDP6979 | Zm00001eb129050 Zm00001eb196170 Zm00001eb038000 Zm00001eb093430 Zm00001eb093440 Zm00001eb128730 Zm00001eb133440 Zm00001eb163460 Zm00001eb129340 Zm00001eb196180 Zm00001eb332620 | overexpression | [112] |
| ZIF2  | carrier protein | PC0099415 GPM828 | Zm00001eb017730 Zm00001eb017760 | overexpression | [113] |
| HMA2  | carrier protein | HMA2 | Zm00001eb226870 | overexpression | [114] |
| HMA4  | carrier protein | HMA3 | Zm00001eb095020 | overexpression | [115] |
| HMA7  | carrier protein | CSU904 | Zm00001eb327860 | overexpression | [116] |
| ZIP1  | carrier protein | ZIP8 | Zm00001eb139810 | overexpression | [117] |
| ZIP8  | carrier protein | ZIP8 | Zm00001eb303800 | knockout | [118] |
| MIPS  | myo-inositol-1-phosphate synthase | MIPS2 | Zm00001eb401220 Zm00001eb276490 Zm00001eb283250 Zm00001eb378070 | knockout | [124] |
| PGK1  | 2-phosphoglycerate kinase | | Zm00001eb391270 Zm00001eb259060 | knockout | [125] |
| ITPK2 | inositol 1,3,4-trisphosphate 5/6-kinase | IDP8938 | Zm00001eb399350 | knockout | [126] |
| IPK1  | inositol 1,3,4,5,6-pentakisphosphate 2-kinase | IDP8938 | Zm00001eb067500 Zm00001eb432760 | knockout | [127] |
| HAD1  | phytase | | Zm00001eb063350 Zm00001eb342820 Zm00001eb399750 | overexpression | [128] |
| PAPHY-A | phytase | PAP2 | Zm00001eb064450 | overexpression | [129] |
| PAP4  | phytase | PAP22 | Zm00001eb048820 | overexpression | [130] |

7. Identification of Maize Potential Gene Resources for Other Secondary Metabolites Content Improvement

In addition to vitamins, there are many secondary metabolites in plants, mainly phenolic compounds. The majority of the phenolic compounds in maize are phenolic acids, such as ferulic, vanillic, caffeic, syringic, synapatic and ϱ-coumaric acids, and polyphenols, including lignins and lignans [131]. Phenolic compounds are essential for plant growth and development and are considered as defensive barriers of plants. However, the detailed mechanism is still unknown, and it is speculated that it plays an antioxidant role [132].

Anthocyanins are flavonoids that confer plant seeds and fruits various colors, from red to purple. They are not just protective agents for plants. Anthocyanins are also used as supplements in health care products to control obesity and diabetes and improve vision and brain function [1]. Many genes related to anthocyanin synthesis have been identified and applied in genetic engineering to improve anthocyanin content in maize. These include many transcription factors that regulate anthocyanin synthesis. For example, GLK1 [133], AN1 [134], AN3 [135] and ANT1 [136] are positive regulators, while GmMYBR [137] is a...
negative regulator for anthocyanin synthesis. A double-stranded RNA binding protein, DRB3 has also been shown to inhibit anthocyanins biosynthesis [138]. Nine homologous genes involved in anthocyanin synthesis were identified from the maize genome (Table 6).

Table 6. List of potential gene resources for enhancing anthocyanin content in maize.

| Genes  | Protein Function       | Maize Orthologs | Gene ID                  | Strategy     | References |
|--------|------------------------|-----------------|--------------------------|--------------|------------|
| GLK1   | transcription factor    | G2 GLK1         | Zm00001eb118900 Zm00001eb371980 | overexpression | [133]      |
| AN1    | transcription factor    | IN1             | Zm00001eb303250          | overexpression | [134]      |
| AN3    | transcription factor    | GIF1            | Zm00001eb056300          | overexpression | [135]      |
| ANT1   | transcription factor    | PL1 C1          | Zm00001eb278680 Zm00001eb373660 | overexpression | [136]      |
| MYBR   | transcription factor    | MYB31 MYB42    | Zm00001eb103730 Zm00001eb202770 | knockout      | [137]      |
| DRB3   | double stranded RNA binding protein | IDP7470 | Zm00001eb102530 | knockout      | [138]      |

8. Expression Patterns of the Putative Nutritional Improvement-Related Maize Genes

For expression analysis of identified potential gene resources, we used 31 different time points seed samples [139] and 6 kernel compartment samples [140] of the B73 inbred line of the RNA-seq data and 16 non-seed tissues of the inbred line SRG200 (Syngenta) of the microarray data [141] downloaded from the Maize eFP database (https://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi?, accessed on 20 February 2022). The expression levels of each gene in different tissue are listed in Table S1, and gene expression heatmaps were generated using the pheatmap package of R software (Figure 5 and Figure S2). Most identified genes are highly expressed in either early seeds or kernels. However, some genes are weakly expressed in both tissues. For instance, GPC1, GPC2, GPC3 and GPC4 that encode glyceraldehyde-3-phosphate dehydrogenases are highly expressed in nucellus at different time points after pollination and in different compartments of kernels. OLE1, OLE3 and OLE4 that encode delta-9 desaturases are weakly expressed in early seeds but highly expressed in different compartments of kernels. This may be because OLEs play a structural role in stabilizing the lipid body during desiccation of the seed by preventing coalescence of the oil (Figure 5). The expression profiles of the putative genes provide important information for the strategy applied to the molecular breeding of nutritionally enriched maize.
Table 6. List of potential gene resources for enhancing anthocyanin content in maize. (A) Expression heatmap of potential gene resources for maize lipid content improvement. (A) Expression heatmap of potential gene resources for maize lipid content improvement from maize nucellus at different time points after pollination. NU0-144 represents the nucellus (embryo sac included) after 0–144 h of self-pollination. (B) Expression heatmap of potential gene resources for maize lipid content improvement from maize kernels. AS, Apical scutellum; End, Endosperm; SAL, Scutellar Alleurone Layer; Emb, Embryo; EAS, Endosperm Adjacent to Scutellum; Per, Pericarp. (C) Expression heatmap of potential gene resources for maize lipid content improvement from 16 maize tissues. Root1,2,3 represent V2, V5 seminal root and adult nodal root, respectively; Leaf1,2,3 represent the 2nd, 4th and 8th leaf, respectively; Ear1,2 represent V8 and V15 ear, respectively; Tassel 1,2,3,4 represent 1 mm, 2 cm, 12 cm and 22 cm tassel, respectively. The color scale bars represent the relative expression level.

9. Discussion

In this review, we summarized genes associated with nutrient biosynthesis, uptake and transport from different plant species, and 246 homologous genes were identified from the maize genome. These genes are promising candidates for improving resistant starch, oil, essential amino acids, vitamins, iron, zinc and anthocyanin levels of maize grains through genome engineering. However, one should also notice that plant phylogeny is complex, and the function of a gene cannot be completely determined from homology alone. Therefore, information regarding maize kernel transcriptome and metabolome would be helpful for the validation of the candidate genes for breeding use. Metabolic profiling of mature maize kernels revealed significant variation among different maize lines. For example, glucose-1-phosphate (G1P) is an intermediate in starch metabolism and was identified as the highest variable metabolite between maize varieties Chang7-2 and Ye478 [142]. UDP-Glycosyltransferase super family proteins catalyze G1P into glucuronate as annotated in the KEGG database. Zm00001eb214570 is an ortholog of AT3G02100 in A. thaliana and encodes a UDP-Glycosyltransferase. The expression of Zm00001eb214570 was undetectable in Ye478 [143] and the level of glucuronate was much lower in Ye478 compared to Chang7-2. Therefore, the accumulation of a high level of G1P in Ye478 probably results from the lack of the expression of Zm00001eb214570. This indicates that metabolomics is generally correlated with transcriptomics. However, one should note that even if the prediction of a gene’s functions is reliable and correct, changes in the metabolic rate of an intermediate process may not have a significant effect on the amount of material synthesized, as precursor materials limit the final content.

The key enzyme SSs for maize starch synthesis are encoded by many homologous genes, which probably have function redundancy. In rice, the repression of genes that
encode isozymes SSI, SSIIa and SSIIIa via RNAi strongly influenced grain development, while repression of the other four SS encoding genes did not show any effect [17]. Another study on rice SS has also suggested that improved grain quality can only be achieved by coordinated downregulation of the expression of SSIIb and SSIIc, indicating a functional redundancy between SSIIb and SSIIc [18].

Previous studies mostly focused on breeding high-yield oil-corn varieties for industrial use. Recently, more and more attention has been paid to improving the nutritional properties of corn. Studies have shown that unsaturated fatty acids are better for human health than saturated fatty acids. In order to reduce the amount of PUFA and increase the amount of oleic acid, a type of MUFA, delta-12 fatty acid desaturase 2 (FAD2), delta-12 fatty acid desaturase 3 (FAD3) and fatty acid elongase 1 (FAE1) are good targets for genetic manipulation. Inhibition of FAD2 [50,51], FAD3 [50] and FAE1 [51] increases the content of MUFA.

Another issue that should be addressed is that many genes have multiple functions and are expressed in various plant tissues and organs. Therefore, applying these genes for nutrient quality improvement using a knockout strategy may also cause serious plant growth and development defects, and the knockdown strategy may be more suitable for such cases. Additionally, protein engineering to generate amino acid substitution mutants instead of knockout may provide another option to solve this problem. This requires knowledge of the working mechanism of the protein and the specific mutation technology.

The mechanisms of vitamin synthesis, mineral absorption and transport are still not fully clear. Many vitamins need to work together, so multivitamins are now advocated. Vitamin absorption also has a great relationship with the cooking method. Studies have shown that the cooking temperature was the decisive factor in the cooking loss of carotenoids in corn, and the boiling and steaming of corn caused it to retain the most nutrients [144]. Using exogenous fertilization seems more straightforward for mineral replenishment, but the cost and problems associated with soil and groundwater contamination make genetic manipulation a better choice. However, the effect of genetic manipulation for mineral content improvement is also related to the cultivar. Although phytic acid is harmful to the absorption of metal ions, it is also the storage form of phosphorus in plants. In order to avoid the effect on phosphorus uptake, the regulation of phytic acid content should be carefully considered.

In addition to modifying plant genes, genetic engineering allows the possibility of introducing genes with special effects from other species such as bacteria into maize. The use of zein promoters that specifically express bacterial \textit{crtB} and \textit{crtI} genes in maize endosperm resulted in a thirty-four times increase in total carotene [145]. The bacterial \textit{lysC} gene encodes an AK, but unlike AK in plants, it is not inhibited by lysine feedback, so when \textit{lysC} was ectopically expressed in tobacco seeds, lysine content was increasingly detected [146]. Similar strategies could also be applied to maize.

Many genes show synergistic effects on a specific biological process. Therefore, overexpression of a series of synthases along the same synthetic pathway may cause more substantial effects than overexpression of one gene alone. Some genes may regulate a synthetic pathway coordinately. For example, when \textit{HGGT} is co-expressed with carotene synthesis genes in sorghum, increased vitamin E can reduce the oxidative degradation of carotene, increase the stability and half-life of carotene and thus increase the carotene content [89].

10. Conclusions

With the increasing population and human nutritional requirements for the daily diet, developing nutrient-rich high-yield crop varieties has become breeders’ primary objective. Biofortification is a good way to improve the nutrient content of plants, and there is much room for application in maize. The development of transcriptomics and metabolomics has provided valuable information for disclosing mechanisms of nutrient compound synthesis. In this review, we summarized the reported genes that are associated with nutrient content.
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from different plant species. Based on the principle that plant homologous genes may have similar functions across species, we identified 246 genes related to nutrient quality from the maize genome and provided physical maps for their chromosome location and detailed expression profiles in early seeds, kernels and non-seed tissues. These genes are potential resources for improving the content of starch, oil, protein, vitamin, mineral and secondary metabolites in maize kernels. Combining the data from transcriptomic, proteomic and metabolomic analyses, constructing maize kernels’ transcriptional, proteomic and metabolic roadmaps will provide a comprehensive relationship between gene regulation and metabolic network, which facilitates gene function validation and future maize breeding with the aim to improve nutritious quality.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11050627/s1, Figure S1: Chromosomal locations of potential gene resources; Figure S2: Expression profiles of potential gene resources for maize; Table S1: Expression levels of 246 maize potential gene resources for nutrient improvement among the different tissues.

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