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Potentiality of RNA Interference Technology in Enhancing the Nutritional Status and Food Value of Plant Species

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ABSTRACT

Bio fortification or enhancement of nutrient levels in plants via genetic engineering aims at alleviating hidden hunger and eradicating micronutrient deficiency-related malnutrition. A number of approaches have been attempted by plant biotechnologists for the same, of which manipulation of alien genes from diverse sources and their consequent integration within the genome of crop plants for expression of desired traits is quite a popular one. RNA interference (RNAi)-mediated gene silencing is an approach that has gained attention and special importance in the last two decades, particularly because of its ability to suppress genes, whose products lead to the formation of various anti-nutrients, allergens and toxins, with appreciable specificity. RNA-induced silencing or RNAi suppresses specific mRNAs in the host plants which leads to inhibition of specific enzymes that regulate many biosynthetic pathways. RNAi uses non-coding siRNAs (small interfering RNA) or miRNAs (micro RNA) to silence the target genes. These non-coding small RNAs have a sequence complementarity to the mRNA to be silenced. The targeted genes generally correspond to the key rate-limiting enzymes in multiple steps of the numerous interconnected pathways of the plant metabolome and hence are carefully chosen for avoiding off-target effects. The major implication of this technology to date has been on staple food crops like rice, wheat and maize, for the enrichment of micronutrients like iron and essential amino acids; however, recent experiments have also targeted plants like coffee, tea and oilseed crops, which equally form inseparable parts of daily food items, worldwide. This review elaborates several such existing examples, highlighting the genes, targeted by RNAi mechanisms, for nutrient and antioxidant enrichment, explores their practical use in daily life and briefly ponders upon the facets of possible initiatives to bring such genetically improved plants to the doorsteps of the masses.

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INTRODUCTION

RNA interference (RNAi) is the method of blocking the function of any gene by the insertion of short ribonucleic acid (RNA) sequences that match part of the sequence of the target gene, preventing the production of the protein, encoded by that gene. The technique was discovered by Andrew Fire and Craig Mello in 1998, when they injected double-stranded RNA (dsRNA) into the worm *Caenorhabditis elegans*, triggering the silencing of genes that had identical sequences as that of the dsRNA. They were subsequently awarded the Nobel Prize for Physiology and Medicine in 2006 for this discovery (Wilson and Doudna, 2013). Cells recognize such dsRNAs as undesirable since they are not formed by the normal genetic mechanisms, and hence such RNAs are cleaved by the cytosolic ribonuclease, Dicer into 21-23 nucleotide-long microRNAs (miRNAs) and small interfering RNAs (siRNAs) with overhanging ends. The siRNA or the miRNA then associates with a pre-RNA-induced silencing complex (pre-RISC), containing an Argonaute protein that distinguishes between the two miRNA or siRNA strands as either sense or antisense, so that the sense strand with the identical sequence as the target gene (passenger strand) can get cleaved, while the antisense strand (guide RNA) gets incorporated to the RISC (Ender and Meister, 2010). The guide RNA-RISC complex then binds to the target complementary RNA sequence, cleaves it at a specific site, using the Argonaute protein, and subsequently degrades it, preventing it from getting translated into a protein. The miRNAs, however, differ from siRNAs in the initial step of their formation, since miRNAs are derived from single-stranded precursor sequences that possess complementary sequences, allowing them to fold back on themselves to form a stem-loop structure (pri-miRNA) at one end, which is cleaved by a nuclear endonuclease complex, Drosha to generate a pre-miRNA with a 3' overhang, which, in turn, is exported to the cytoplasm for Dicer to act on (Wilson and Doudna, 2013). In plants, the success of RNAi-mediated silencing depends on a number of factors, primarily on the architecture of the RNAi constructs. They usually have a spacer or an intron sequence between an inverted repeat, resulting in stem-loop structures like the miRNAs, which are here called hairpin RNAs (hpRNAs); these RNAi constructs can, in turn, be driven by either a strong constitutive promoter, such as the CaMV35S (dicots) or the maize ubiquitin1 (monocots) or tissue-specific promoters. The method of transformation is another crucial factor determining the efficiency of the technique. Although *Agrobacterium*-mediated transformation is the usual method of choice, direct introduction of RNAi vectors via particle bombardment and electroporation has also been employed (Saurabh et al., 2014). Hidden hunger, referring to overall poor quality of nutrition, not only causes a lack of nourishment or malnutrition, but also has collateral effects in impairment of the mental and physical development of children and adolescents, resulting in lower IQ, stunting, blindness, gradual deterioration of immune function and curbing of productivity with a consequent reduction in work capacity. The ill-effects of hidden hunger especially affect the socio-economically challenged population of the world with limited awareness and education of the various aspects of a truly nutritious diet, relying mostly on the meager staple diet for sustenance and satisfaction of hunger (Roychoudhury, 2020). As such, many techniques, ranging from pharmaceutical supplementation and industrial fortification to dietary supplementation have been attempted to alleviate human micronutrient deficiency. However, the sheer expense and inaccessibility of such sophisticated supplements serve little to the impoverished and underprivileged. Therefore, the unanimous solution to the problem has been

the enhancement of the bioavailable micronutrients in food crops through the strategy called 'bio fortification', precisely defined as "fortification in the field rather than in the factory". Bio fortification can be carried out by several techniques, such as agronomic bio fortification, improvement of plant varieties via conventional breeding, and genetic engineering (Sharma et al., 2017).

Among the methods of genetic engineering, overexpression of a particular micronutrient by manipulating genes in the metabolic pathways, involved in its biosynthesis and catabolism, is a primary and major approach. A complementary approach involves the reduction or silencing of the genes involved in the synthesis of 'anti-nutrients, allergens, etc. which inhibit the absorption of nutrients or render toxicity to food crops. This second approach is exemplified in plants, mostly by the use of genetic engineering techniques such as RNAi and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9-mediated gene silencing (Ali *et al.*, 2010). This review focuses on some of the success stories in implementing RNAi technology in improving the levels of different nutrients and ensuring food safety in different plant species.

Iron bio fortification in rice

The average daily allowance of iron for healthy males is 8 mg between 19 and 50 years of age, and 18 mg of iron for healthy females belonging to the same age group. The actual average daily intake of iron from food sources, however, is not adequate and often leads to varying stages of iron deficiency, especially in pregnant and pre-menopausal women. Characterized by World Health Organization (WHO) as one of the leading risk factors for worldwide disease, iron deficiency not only leads to anemia (Iron Deficiency Anaemia or IDA), but also disturbances of the gastrointestinal system, fatigue, weakness and impairment of normal immune function, and in its most severe stage, even to compromised neurocognition. The need for iron bio fortification is thus obvious and evident, and rice being one of the major staple cereals in South East Asia, Latin America and some parts of Africa, is a suitable target crop for the same. The importance of bio fortification specific to milled rice is further highlighted by the fact that post-harvest processing in brown rice has been found to reduce the iron content by around 4.8 times, which further decreases another 2.0-fold in the polished consumable grains. There are several approaches of iron bio fortification in rice, such as endosperm-specific expression of the iron storage protein ferritin, overexpression of the nicotinamide synthase gene, *NAS1*, overexpression of iron transporters like IRT1, which target iron storage, chelation and transport at various levels in rice (Majumder et al., 2019).

RNAi technology has been used in the iron bio fortification of rice by lowering the phytic acid content. Phytic acid is an antinutrient, since it prevents iron utilization and solubilization in plants due to its strong cation-chelating ability. Abbreviated as IP6 due to its chemical name myo-inositol-1, 2, 3, 4, 5, 6-hexakisphosphate, phytic acid accumulates in the aleurone layer of cereals, except for maize, as mixed salts called phytate. Phytate is a strong chelator of various cations like Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} due to the six negatively charged phosphate groups. This reduces the bioavailability of these micronutrients in the plant. Various enzymatic steps have been targeted in the phytic acid biosynthetic pathway to reduce its concentration in rice. The first enzyme in the biosynthetic pathway of phytic acid is myo-inositol-3-phosphate synthase (MIPS), the corresponding gene for which has been targeted for silencing. Though silencing of *MIPS* gene decreased the phytic acid levels indirectly by reducing the synthesis of myo-inositol, it exerted adverse effects on plants by making them susceptible to abiotic stress, since myo-inositol is an important osmolyte. Therefore, the RNAi approach was used to

silence a different gene encoding the last enzyme, inositol-1, 3,4,5,6 pentakisphosphate 2-kinase 1 (IPK1) in the phytic acid biosynthetic pathway. *IPK1* gene was silenced using a seed-specific *oleosin18* (*Ole18*) promoter, and the transgenic rice showed a 3.9-fold down-regulation in *IPK1* transcripts in transgenic seeds, correlating to a 1.8-fold greater iron accumulation in the endosperm, without affecting the normal growth and development of the plant (Ali et al., 2013).

Manipulating the level of glutelin in rice and gliadin in wheat

Glutelin is a major seed-storage protein in rice, making up 60% of the total endosperm protein content. It is encoded by a multigene family, containing two highly similar subfamilies, *GluA* and *GluB.* It is synthesized as a 57 kDa precursor which is cleaved to 22-23 kDa basic subunit and 37-39 kDa acidic subunit. A dominant mutation, *low glutelin content 1* (*Lgc1*) reduces glutelin content in rice grains. In *Lgc1* homozygous lines, a tail-to-tail inverted repeat is formed by a 3.5 Kbp deletion between the two highly similar *glutelin* genes. This results in a dsRNA which can potentially induce gene silencing. Transgenic analysis of the *lgc1* candidate region using reporter gene assay detected small interfering RNAs, supporting the hypothesis that *lgc1* can suppress *glutelin* expression via RNAi. In the *lgc 1* mutant line, a reduction of glutelin content was seen along with an increase in the levels of other seed storage proteins like prolamin. Such induction of prolamin synthesis was not found to be specific for LGC 1, but was simply a compensation for glutelin reduction. Prolamins accumulated in the protein body I, whereas glutelin accumulated in the protein body II. Since protein body I is barely digested in humans, the presence of LGC 1 can help in producing low-protein rice cultivar (Kusaba et al., 2003). This is highly beneficial for kidney disease patients, who are prescribed a restricted protein intake and cultivars harboring LGC 1 are now being used in low-protein diet therapy.

In bread wheat cultivar 'Bobwhite' lines, RNAi approach was used to down-regulate gamma-gliadin which caused an increase in glutenin content, along with a slight increase in the total protein content (Gil-Humanes et al., 2012). Reduced gliadin wheat is an alternative for consumers suffering from celiac disease. Moreover, reduced gliadin flour has high nutritional property, since it has increased lysine content.

Lysine biofortification in rice and maize

Lysine is an essential amino acid (EAA) that serves as an important source of energy and nutrition for humans and livestock. However, cereals such as rice and maize are deficient in lysine, which, being the limiting EAA, also restricts the absorption and utilization of other amino acids, leading to diseases like kwashiorkor or marasmus in cases of severe lysine deficiency and resultant protein deficiency. Thus, the biofortification of rice with lysine is a major interest of plant breeders. Of the several enzymes that have been targeted in the biosynthetic pathway of lysine, aspartate kinase (AK) and dihydrodipicolinate synthase (DHDPS) are particularly noteworthy, since mutant forms of these enzymes which are insensitive to lysine feedback inhibition have shown to increase lysine levels considerably. In addition, another key enzyme playing a major regulatory role in lysine catabolism is lysine ketoglutaratereductase/saccharopine dehydrogenase (LKR/SDH), which, if down-regulated or inhibited, has been shown to stimulate accumulation of lysine, preventing its breakdown. Further, overexpression of maize feedback-insensitive *DHDPS* coupled with an *LKR/SDH* RNAi construct in rice showed a 60-fold increase in free lysine level in matured seeds of transgenic rice, as compared to the wild-type seeds (Lee *et al.*, 2001; Frizzi et al., 2008). In this regard, three constructs, 35S, Ri and 35R were respectively designed to (i) allow constitutive expression of the bacterial lysine feedback-insensitive

AK and *DHDPS* genes, both driven by the CaMV35S promoter, (ii) inhibit expression of the *LKR/SDH* gene by LKR-RNAi construct under the endosperm-specific rice *glutelin* (*Gt1*) promoter, and (iii) express the combined transgenes of constructs for functions (i) and (ii) (Yang et al., 2021).

In maize, on the other hand, *opaque 2 (o2)* is a mutant with an opaque and floury kernel where the basic leucine zipper transcription factor O2, which regulates endosperm-specific genes like α-zein (22 kDa) and *LKR/SDH*, is mutated. The resultant reduced levels of α-zein were however compensated by other non-zein lysine-rich proteins, thus increasing the net lysine content. RNAi-mediated silencing of lysine-poor α-zeins has been shown to mimic the opaque kernel phenotype of the *o2* mutant, along with high lysine content, pointing to the fact that decrease in α-zein is directly correlated with higher lysine content, thereby enhancing its nutritional quality (Houmard et al., 2007).

Increasing amylose content in wheat and sweet potato

Wheat is a major staple crop that forms an important source of carbohydrates, proteins, fats, vitamins and minerals, contributing to a healthy human diet, with starch being the major component of the wheat kernel. Starch is composed of amylopectin (70-80% of dry weight), a highly branched polysaccharide, together with amylose (20-30% of dry weight), a linear chain of D-glucose molecules with few branches, for 70-80% and 20-30% of the starch dry weight, respectively. While amylopectin is easily digested by humans and other mammals, amylose tends to form complexes that are resistant to digestion, mimicking the dietary fiber. When cooked food cools down, amylose molecules re-associate rapidly, resisting digestion, whereas amylopectin molecules re-associate slowly and get more readily digested. Therefore, starch with higher amylose content is called resistant starch (RS), and increased consumption of RS is shown to be associated with several health benefits, such as the lower risk of type II diabetes, obesity, cardiac diseases, and colorectal cancers. A reduction in starch digestion in the small intestine also leads to a decrease in the rate of entry of glucose into the bloodstream, which in turn lowers the insulin demand and the glycemic index of the food items consumed. Besides, the undigested starch moves to the large bowel from the small intestine, underlining their fecal bulking and laxative action, and is finally converted to short-chain fatty acids by fermentation, which is shown to be associated with increased satiety (Williams, 1995). Therefore, engineering wheat to have a higher percentage of inefficiently-digested amylose is of special interest, given its enhanced nutritional and health-promoting roles. RNAi-mediated gene silencing has been used to suppress the expression of two different isoforms of starch-branching enzyme of class II, viz., Sbeiia and Sbeiib in the durum wheat endosperm, to increase the amylose content by decreasing amylopectin biosynthesis. Hairpin-RNA (hpRNA) molecules, corresponding to the sequences of *SBEIIa* and *SBEIIb* were expressed in inverted repeats in wheat, under the endosperm-specific *glutenin* (high-molecular-weight Dx5 subunit gene) promoter, which resulted in more than 70% increase in amylose content. Rats fed with this high amylose wheat grain as a whole meal showed improved bowel function in comparison to standard wholemeal wheat, further emphasizing its beneficial role in human health due to its enhanced resistant starch content (Regina *et al*., 2006; Sestili *et al*., 2010). Similar RNAi techniques were applied in the case of sweet potato (*Ipomoea batatas*) which yielded higher amylose content. The storage roots of sweet potato *(Ipomoea batatas*) contain starch which is made up of unbranched linear amylose (10-20%) and branched amylopectin, constituting the rest. The starch branching enzymes which are responsible for producing branched amylopectin are of two classes –

class A (potato SBEII) and class B (potato SBEI). A dsRNA interference vector was constructed based on the cDNA of sweet potato SBEII (IbSBEII) and introduced in the sweet potato genome via *Agrobacterium*-mediated plant transformation (Shimada et al., 2006).

Lowering the level of sucrose phosphate in potato

The two enzymatic steps of sucrose biosynthesis involve the catalysis of the synthesis of sucrose-6-phosphate (Suc6P) by sucrose-phosphate synthase (SPS), followed by hydrolysis of Suc6P by sucrose phosphatase (SPP) to yield sucrose and inorganic phosphate (Pi). Accumulation of glucose and fructose due to sucrose hydrolysis occurs by a process called hexogenesis when potato tubers are stored at 4°C. This process is called 'cold sweetening', which causes the browning of the potato, imparts a bitter taste to it, thereby deteriorating the nutritional and commercial value. During potato chip and French fry productions, the reducing sugars in potato react with free amino acids in a non-enzymatic Maillard reaction to produce brown- to the black-pigmented product along with carcinogenic acrylamide that is not acceptable to the consumers. Reducing sugars and asparagine are the two major substrates for acrylamide formation in processed potato products. CaMV35S promoter-driven hairpin RNAi constructs, containing part of the coding region of the tobacco *NtSPP2* gene were used by Chen *et al.* (2008) to reduce *SPP* expression in transgenic potato tubers. Suc6P was reported to be accumulated in RNAi mediated-*SPP*-silenced potato tubers when stored at 4°C, along with decreased sucrose accumulation. It was revealed that cold-induced expression of vacuolar invertase (VINV) was blocked in *SPP*-silenced tubers. This further explained the presence of a reduced sucrose-to-hexose conversion. Further silencing of the *VINV* gene through RNAi showed dramatically reduced acid invertase activity and no reducing sugar was accumulated during cold storage of potato.

Improving starch accumulation in *Arabidopsis* and maize

Starch phosphorylation and dephosphorylation are the important events regulating starch accumulation. Glucan water dikinase (GWD) adds phosphate to starch, while phosphoglucan phosphatase (SEX4) removes these phosphates. RNAi constructs were introduced in *Arabidopsis thaliana* to reduce the expression of *AtGWD* and *AtSEX4*. The starch build-up was manipulated with ethanol-inducible and senescence-induced gene promoters. Ethanol induction of RNAi lines lessened transcript accumulation for *AtGWD* and *AtSEX4* by 50%. At the end of the dark period, the transgenic lines exhibited seven times more starch, but they exhibited similar growth rates and total biomass, as the wild-type plants. Likewise, RNAi construct against *AtGWD*-homologous gene from maize under the constitutive *ubiquitin* promoter elevated starch content in maize with no impact on total plant biomass (Weise et al., 2012).

Increasing grain yield

RNAi-mediated suppression of *GA20 oxidase* (controlling gibberellin biosynthesis) gene resulted in semi-dwarf plants from taller rice variety, QX1. The transgenics showed an increase in panicle length, increased number of seeds per panicle and higher grain weight (Qin *et al.*, 2013). OsSPL14 (Squamosa promoter binding protein like14) was reported to be regulated by microRNA (miRNA), OsmiR156 in rice (Jiao et al., 2010). A point mutation in OsSPL14 perturbs OsmiR156-directed regulation of OsSPL14, generating an 'ideal' rice plant with reduced tiller number, increased lodging resistance and enhanced grain yield.

Increasing shelf life of tomato

1-Aminocyclopropane-1-carboxylate (ACC) oxidase catalyzes the oxidation of ACC to ethylene, a phytohormone that plays an important role in the ripening process of tomato fruit. A double-stranded (ds) RNA

expression unit of tomato ACC oxidase was introduced into tomato cultivar Hezuo 906. The sense and antisense configurations of DNA fragments were linked with 1,002 bp or 7 nt artificially-synthesized fragments, respectively, and placed under the control of a modified CaMV35S promoter. With respect to the construct with the 1,002 bp linker, the severity of the phenotypes indicated that 72.3% of the transformed plants had non-RNA interference, about 18.1% had semi-RNA interference, and only 9.6% had full-RNA interference. However, when the construct with the 7 nt linker was used for transformation, the results were 13.0%, 18.0%, and 69.0%, respectively, indicating that the short linker was more efficient in RNAi of transgenic tomato plants. Transgenic tomato plants were produced where *ACC oxidase* gene was shut down, producing fruits that released traces of ethylene and had a prolonged shelf life of more than 120 days (Xiong et al*.,* 2004).

Enhancing flavonoid and carotenoid levels

The downregulation of carotenoid cleavage dioxygenases (CCD) can increase the overall carotenoid content in rice seeds and leaves by preventing the cleavage or degradation of carotenoids, the precursor for Vitamin A. It was found that CCD4a-suppressed variants of rice showed a greater accumulation of carotenoids in seed endosperm and leaves. Among the three *CCD* genes in rice, the *CCD1* expression is generally higher in leaves, *CCD4a* is expressed in young seedlings and to some extent in seeds (lesser expression in leaves, as compared to *CCD1*), and *CCD4b* expression is generally up-regulated in seeds during later stages. Three different RNAi vector constructs were made, using cDNA sequences for each of the *CCD* genes, including untranslated regions. *OsCCD4a* suppression lines showed the maximum overall increase in carotenoid levels (Ko et al., 2018).

RNAi approach was used to enhance the carotenoid and the flavonoid content of tomato (*Lycopersicon esculentum*), both of which are highly beneficial as antioxidants for human health. RNAi was used to suppress *Tomato DE-ETIOLATED 1* (*TDET1*, an endogenous photomorphogenetic regulatory gene) with the help of DET-1 derived inverted repeat constructs which were driven by three different fruit-specific promoters. The transgenic tomatoes showed specific degradation of *TDET1* transcripts, along with an increase in carotenoid and flavonoid content (Davuluri *et al*., 2005). Similarly, RNAi has been used to down-regulate the expression of the *lycopene epsilon cyclase* (*ε-CYC*) gene to enhance the carotenoid content of rapeseed (*Brassica napus*). The transgenic *Brassica* seeds showed increased levels of β-carotene, zeaxanthin, violaxanthin and lutein (Yu et al., 2007). The β-carotene hydroxylase (BCH) enzyme converts β-carotene to zeaxanthin in potato (*Solanum tuberosum*). Silencing of *BCH* gene through RNAi using GBSS (granule bound starch synthase) promoter led to increase in two health-promoting carotenoids, β-carotene and lutein, in potato (Eck et al., 2007). The *SlNCED 1* gene encodes for 9-cis-epoxycarotenoid dioxygenase (NCED) which is the key enzyme in abscisic acid (ABA) biosynthesis in tomato fruit. This gene was silenced via RNAi under fruit-specific *E8* promoter that led to lowering in ABA level due to a significant reduction in the NCED activity. Thus, carbon that is normally channelized to free ABA metabolic pathway during ripening is partially blocked or 'backlogged', and on the contrary, is diverted to the carotenoid pathway in the RNAi lines, causing an increase in the assimilation and accumulation of lycopene and β-carotene (Sun et al., 2012).

Increasing the levels of ascorbic acid in tomato

Ascorbic acid/Vitamin C helps in the enhancement of iron absorption, boosting immunity, and decreasing blood cholesterol among the various functions. The deficiency of vitamin C is known to cause scurvy. The primary enzymes for the oxidation of

ascorbate in plants are ascorbate peroxidase (*APX*) and ascorbate oxidase (*AO*), which oxidize ascorbate to monodehydroascorbate. The levels of ascorbate can be increased in fruits by suppressing their oxidation. RNA-interference vector was constructed using a 597 bp fragment from the 5' end of mitochondrial *SlAPX* gene of tomato, cloned into the vector, which was then transformed into *Agrobacterium tumefaciens* through electroporation. The transgenic plants thus produced showed a 1.4-2.2-fold increase in the ascorbic acid levels in tomato, as compared to the wild-type control (Zhang *et al.,* 2011).

Manipulating the levels of fatty acids in cotton seed, soybean and flax

Cotton (*Gossypium hirsutum*) is one of the most important economic crops for the production of fiber, but it is also the sixth-largest source of vegetable oil in the world. The composition of cotton seed oil is such that it has a relatively high level of palmitic acid (C16:0), which is a saturated fatty acid, stabilizing the oil and making it suitable for frying at high temperature. Moreover, hydrogenation of cotton seed oil to produce solid margarine hard stock also leads to the formation of trans-fatty acids. Both saturated fatty acids and trans-fatty acids have been shown to possess equivalent properties of raising the undesirable low-density lipoprotein (LDL) cholesterol and lowering the desirable high-density lipoprotein (HDL) cholesterol.

Due to growing awareness about the ill effects of LDL and its correlation with coronary heart diseases, there is an increasing trend in the use of favoring oils, rich in unsaturated fatty acids such as oleic acid (C18:1), and low in palmitic acid, but rich in stearic acid (C18:0). Such oils are both nutritionally beneficial and provide the required function without the need for hydrogenation. The major enzymes controlling the synthesis and catabolism of such desired fatty acids are desaturases, especially stearoyl-acyl-carrier protein (ACP) Δ9-desaturase (encoded by *ghSAD-1* in cotton seed), which converts stearic acid to oleic acid, and microsomal ω6-desaturase or Δ12-desaturase (encoded by *ghFAD2-1* in cotton seed), which converts oleic acid to linoleic acid.

RNAi-mediated seed-specific silencing of *ghSAD-1* led to the accumulation of stearic acid, resulting in high-stearic (HS) cotton seed oil, while silencing of *ghFAD2*-1 resulted in the accumulation of oleic acid, leading to high-oleic (HO) cotton seed oil. Cotton cv Coker 315 was transformed with constructs, containing seed-specific soybean lectin promoter, and *ghSAD-1* or *ghFAD2-1* cDNA clone in the inverted repeat to generate hpRNA-mediated gene silencing. It was observed that stearic acid increased substantially to 40%, compared to the normal levels of 2-3%, and oleic acid content soared to as high as 77% in contrast to 15% in non-transgenic seeds. These results were also concomitant with a significant reduction in palmitic acid levels to only 12% of the total fatty acids in double homozygous F2 plants that carried both the gene silencing constructs, corresponding to *ghSAD-1* and *ghFAD2-1*, obtained on intercrossing of stable lines expressing the HS and HO traits (Liu et al. 2002; Segal et al. 2003).

Consumption of α-linolenic acid (18:3) was found to be unhealthy for human beings. Improvement of soybean oil flavor and stability required a reduction in its α-linolenic acid levels. The *omega-3 fatty acid desaturase* (*FAD3*) gene family comprising *GmFAD3A*, *GmFAD3B* and *GmFAD3C* produces the FAD3 enzyme which converts linoleic acid (18:2) to α-linolenic acid in the polyunsaturated fatty acid pathway. A 318-nucleotide long conserved sequence, common to all the three gene family members, was used as an inverted repeat in the RNAi expression cassette placed under a seed-specific *glycinin* promoter. The transgenic line was found to contain 1-3% of α-linolenic acid, as compared to 7-10% in

non-transgenic soybean seed. This siRNA-mediated silencing of *FAD3* was also seen to be stably inherited in transgenic soybean lines. Marked reduction in α-linolenic acid by this approach enhanced the agronomic value of the seed (Flores et al. 2008). Flax possesses two isoforms of FAD2 enzymes that convert monounsaturated oleic acid to polyunsaturated linoleic acid. RNAi approach was used to silence both the *FAD2* genes simultaneously in flax that led to high levels of oleic acid, instead of linoleic acid and this trait remained stable across several generations (Dar et al., 2017).

Enhancing secondary metabolites for nutraceutical and pharmaceutical applications

Squalene epoxidase enzymes (encoded by the genes *pgSQE1* and *pgSQE2*) catalyze the rate-limiting step in the biosynthesis of phytosterol and triterpenoid saponin. RNA interference of *PgSQE1* in transgenic *Panax ginseng* completely
suppressed *PgSQE1* transcription.
Concomitantly, the interference of *PgSQE1* resulted in the reduction of ginsenoside production. Interestingly, silencing of *PgSQE1* in the roots of RNAi-engineered plants strongly up-regulated *PgSQE2* and *PNX* (cycloartenol synthase) and resulted in enhanced phytosterol accumulation. These results indicated that the expression of *PgSQE1* and *PgSQE2* were regulated differently and that *PgSQE1* regulates ginsenoside biosynthesis, but not that of phytosterols in *P. ginseng (Han* et al*., 2010)*.
Artemisinin is an anti-malarial drug obtained from *Artemisia annua*, but the content of artemisinin is low. Suppressing the expression of SQS (squalene synthase), the key enzyme of sterol pathway (a pathway competitive with that of artemisinin biosynthesis) using a hairpin-RNA-mediated RNAi technique was attempted, which showed desirable results in increasing artemisinin content in the transgenics (Zhang et al., 2009). In another work, DNA-encoded hairpin RNA-mediated suppression of the gene encoding morphinan pathway enzyme, salutaridinol 7-*O*-acetyltransferase (SalAT) in opium poppy resulted in the novel accumulation of the pharmacologically important alkaloid, salutaridine at up to 23% of total alkaloid; this alkaloid was not detectable in the parental genotype (Allen *et al*. 2008). Earlier, Allen et al*.* (2004) also reported high-yield accumulation of the non-narcotic alkaloid, reticuline, at the expense of morphine, codeine, oripavine and thebaine, by silencing of codeinone reductase (COR) in opium poppy, *Papaver somniferum*, using a chimeric hairpin RNA construct, designed to silence all members of the multigene *COR* family through RNAi. Since reticuline is quite an early precursor of codeinone, opium poppy was metabolically engineered by this approach to prevent entry into the morphine synthesis branch.

Lowering caffeine content in coffee and tea

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid found in tea and coffee plants. It is most commonly used as a stimulator of the central nervous system; however, studies indicated several adverse effects of caffeine as well, such as palpitations, gastrointestinal disturbances, anxiety, high blood pressure and insomnia. This led to more interest in recent times in the consumption of decaffeinated or 'decaf' beverages, particularly coffee (Ashihara and Crozier 2001). Transgenic coffee plants have been engineered, targeting several methyltransferases in the caffeine biosynthetic pathway. First, xanthosine is methylated by xanthosine methyltransferase (XMT), producing 7-methylxanthosine. Next, its ribose residue is removed and the resulting 7-methylxanthine is methylated by 7-N-methylxanthine methyltransferase (MXMT or theobromine synthase), producing 3, 7-dimethylxanthine (theobromine) as the product. Theobromine in turn is methylated by 3, 7-dimethylxanthine methyl ransferase (DXMT or caffeine synthase) to caffeine (1, 3, 7-trimethylxanthine). Therefore, genes

encoding MXMT and DXMT enzymes were used as targets for RNAi-mediated gene silencing. The cDNAs encoding the three enzymes were cloned and designated as *CaXMT1, CaMXMT1* and *CaDXMT1* and these were used to construct dsRNA which could lead to RNAi-mediated gene silencing in *Coffea arabica* and *C. canephora* under a constitutive CaMV35S promoter. The transformed tissues showed reduced transcript levels corresponding to all the three enzymes, concomitant with a reduction in the levels of theobromine and consequently, caffeine, with 50-85% lowering of theobromine for the non-transgenics, 100% decaffeination in embryonic tissues and 70% decaffeination in plantlets (Ogita et al., 2004).

Alleviation of the adverse effects of caffeine on human physiology is a promising impetus for not only generating transgenic coffee plants, but also transgenic tea, especially green tea, for its added health benefits. Suppression of caffeine by RNAi-mediated gene silencing by targeting the same three enzymes as mentioned above was undergone in tea, resulting in 44-61% reduction in caffeine and 46-67% decrease in theobromine contents, as compared to the controls (Mohanpuria et al. 2011).

Lowering the level of sinapate esters

Removal of sinapate ester can improve the canola oil seed value by decreasing the overall polyphenol content and increasing the nutritional value. Hüsken et al. (2005) used the RNAi method to down-regulate the *sinapate ester synthase* genes. The main biosynthetic gene in the sinapyl ester pathway is UDP-glucose: sinapate glucosyltransferase (BnSGT1). The dsRNA construct was made with *BnSGT1* under the influence of seed-specific napin promoters. An overall 76% decrease in sinapate ester levels was noted in transgenic canola seeds. Removal of sinapate esters enhanced the flavor of canola seeds, along with an increase in the content of resveratrol that is known to reduce heart disease, arteriosclerosis and cancer. However, no other properties like fatty acid content and oil content were influenced by this approach. Moreover, the heritability of this trait ensured constant maintenance of nutritional and agronomic status.

Removal of toxic compounds in edible plants

Cassava is a major staple food crop in many tropical countries. However, it contains toxic cyanogenic glycosides within the tuber. Antisense down-regulation of genes encoding cytochrome P450 enzymes, CYP79D1 and CYP79D2, which catalyze the first step of the synthesis of linamarin and lotaustralin, generated transgenics with more than 90% reduction of cyanogenic glycosides in the tubers (Siritunga and Sayre, 2003). In another work, RNAi technology was successfully used to down-regulate the expression of *Mald1*, which leads to the production of a prominent allergen in apples. Transgenic apple harboring intron-spliced hpRNA construct showed an approximately10-fold reduction in *Mald1* expression in the leaf. This interference event also suppressed the synthesis of sorbitol that affects the quality of fruit via starch accumulation and acid-sugar content (Teo et al., 2006). RNAi approach was also used to simultaneously silence two *asparagine synthase* genes (*StAs1* and *StAs2*) to generate potatoes with reduced asparagine, one of the main precursors for the neural toxin, acrylamide (Zhu *et al*., 2016). Subsequent genetic manipulation led to the creation of transgenic lines with reduced levels of toxic steroidal glycoalkaloids by silencing the gene encoding the enzyme, sterol side chain reductase 2. People consuming *Lathyrus sativus* (a leguminous crop), suffer from a paralytic disease called lathyrism, due to the presence of a neurotoxin called β-oxalylaminoalanine-L-alanine (BOAA). The gene responsible for the production of BOAA was therefore suppressed using RNAi method (Zhang et al. 2016). Sunilkumar et al. (2006) observed that

transgenic cotton plants expressing RNAi construct of the *δ-cadinene synthase* gene (encoding the enzyme responsible for the synthesis of the toxic compound, gossypol), when fused to a seed-specific promoter, caused reduction in the gossypol content. Lyc e3, an allergen present in tomato has been lowered through RNAi. Lyc e 3 encodes a hydrophilic non-specific lipid transfer protein that directs specific intermembrane lipid transfer. Human IgE antibody shows a strong reactivity to Lyc e3 which leads to IgE sensitization and causes allergenic reactions. Two cDNAs, LTPG1 and LTPG2, with high sequence homology to the N-terminal sequence of Lyce 3 were used for dsRNAi construct. To assess the allergenic potential of Lyc e 3-deficient tomato fruits, the histamine released from sensitized human basophils stimulated with transgenic and parental lines were measured, when a strong reduction in the release of histamine was observed from the basophils, challenged with transgenic tomatoes, as compared with the control plants (Le et al., 2006).

CONCLUSION

Food security in the wake of an ever-increasing world population is a challenge that has no single solution to date. RNAi-mediated gene silencing has led us to understand and discover valuable targets to enhance micronutrient availability in a variety of plants, some of which have been exemplified in this review. The issue of "off-target" silencing by RNAi has also been addressed in plants by the use of tissue-specific and inducible promoters, allowing targeted silencing only in specific tissues. It is a favoured technology since it is cost-effective, producing RNAi inducers throughout the life of the plant. RNAi-mediated gene silencing suffers from certain limitations as well. It may cause chromatin modification which leads to the formation of heterochromatic regions. Such alterations can be heritable and may have ill effects, thus compromising biosafety. Hence, plants produced via RNAi must be assessed for potential risks involved, concerning food safety and its effect on the environment. Complete knockouts or gene silencing may not always be achieved by employing RNA silencing techniques. Furthermore, many endogenous plant promoters are resistant to siRNA-directed transcriptional silencing. Such siRNA or long dsRNA produced by the current RNAi vectors trigger RNA-dependent protein kinase (PKR) pathways in plants which could activate plant stress response and cause certain side effects. A solution to this problem could result from the development of the next-generation RNAi vectors with characteristics of miRNA vectors. The miRNA structures have been selectively evolved to evade the PKR pathway and show no adverse effects other than its programmed role. In conclusion, while on the one hand, further research efforts for plant nutrient enhancement through RNAi are necessary, it is also essential to generate awareness among the masses and make the transgenic products reach them after rigorous assessment of biosafety, allergenicity and toxicity, so that they can be practically put to use, to assure nutritional security for all, rather than remaining a distant elusive goal.

REFERENCES

Ali, N.; Datta, S.K.; Datta, K. RNA interference in designing transgenic crops. *GM Crops* 2010, 1(4), 207-213.

Ali, N.; Paul, S.; Gayen, D.; Sarkar, S.N.; Datta, S.K.; Datta, K. RNAi mediated down regulation of myo-inositol-3-phosphate synthase to generate low phytate rice. *Rice* 2013, 6, 12.

Allen, R.S.; Miller, J.A.C.; Chitty, J.A.; Fist, A.J.; Gerlach; W.L. et al. Metabolic engineering of morphinan alkaloids by over-expression and RNAi suppression of salutaridinol 7-*O*-acetyltransferase in opium poppy. *Plant Biotech. J.* 2008, 6, 22-30.

Allen, R.S.; Millgate, A.G.; Chitty, J.A.; Thisleton, J.; Miller, J.A.C. *et al.* RNAi-

mediated replacement of morphine with the nonnarcotic alkaloid reticuline in opium poppy. *Nat. Biotechnol.* 2004, 22(12), 1559-1566.

Ashihara, H.; Crozier, A. Caffeine: a well-known but little mentioned compound in plant science. *Trends Plant Sci.* 2001, 6(9), 407-413.

Chen, S.; Hajirezaei, M.R.; Zanor, M.I.; Hornyik, C.; Debast, S. RNA interference-mediated repression of sucrose-phosphatase in transgenic potato tubers (*Solanum tuberosum*) strongly affects the hexose-to-sucrose ratio upon cold storage with only minor effects on total soluble carbohydrate accumulation. *Plant Cell Environ.* 2008, 31(1), 165-176.

Dar, A.A.; Choudhury, A.R.; Kancharla, P.V.; Arumugam, N. The *FAD2* gene in plants: occurrence, regulation, and role. *Front. Plant Sci.* 2017, 8, 1789.

Davuluri, G.R.; Tuinen, A.; Fraser, P.D.; Manfredonia, A.; Newman, R.; Burgess, D. *et al.* Fruit-specific RNAi mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nat. Biotechnol.* 2005, 23, 890-895.

Eck, J.V.; Conlin, B.; Garvin, D.F. *et al.* Enhancing beta-carotene content in potato by RNAi-mediated silencing of the *beta-carotene hydroxylase* gene. *Amer. J Potato Res.* 2007, 84, 331.

Ender, C.; Meister, G. Argonaute proteins at a glance. *J. Cell Sci.* 2010, 123 (Pt 11),1819-1823.

Flores, T.; Karpova, O.; Su, X.; Zeng, P.; Bilyeu, K.; Sleper, D.A.; Nguyen, H.T.; Zhang, Z.J. Silencing of *GmFAD3* gene by siRNA leads to low alpha-linolenic acids (18:3) of *fad3*-mutant phenotype in soybean [*Glycine max* (Merr.)]. *Transgenic Res.* 2008, 17, 839–850.

Frizzi, A.; Huang, S.; Gilbertson, L.A.; Toni, A.; Armstrong, T.A.; Luethy, M.H.; Malvar, T.M. Modifying lysine biosynthesis and catabolism in corn with a single bifunctional expression/silencing transgene cassette. *Plant Biotechnol. J.* 2008, 6, 13–21.

Gil-Humanes, J.; Pistón, F.; Altamirano-Fortoul, R.; Real, A.; Comino, I.; Sousa, C. *et al.* Reduced-Gliadin Wheat Bread: An Alternative to the Gluten-Free Diet for Consumers Suffering Gluten-Related Pathologies. *PLoS ONE* 2014, 9(3), e90898.

Han, J-Y.; In, J-G.; Kwon, Y-S.; Choi, Y-E. Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squaleneepoxidase gene in Panax ginseng. Phytochemistry 2010, 71(1), 36-46.

Houmard, N.M.; Mainville, J. L.; Bonin, C.P.; Huang, S.; Luethy, M.H.; Malvar, T.M. High-lysine corn generated by endosperm-specific suppression of lysine catabolism using RNAi. *Plant Biotechnol. J.* 2007, 5, 605–614.

Hüsken, A.; Baumert, A.; Milkowski, C.; Becker, H.C.; Strack, D.; Möllers, C. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (*Brassica napus* L.). *Theoretical and Applied Genetics* 2005, 111(8), 1553-1562.

Jiao, Y.; Wang, Y.; Xue, D.; Wang, J.; Yan, M. *et al.* Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. *Nat. Genet.* 2010, 42(6), 541-544.

Ko, M.R.; Song, M.H.; Kim, J.K.; Baek, S.A.; You, M.K.; Lim, S.H.; Ha, S.H. RNAi-mediated Suppression of Three Rice Carotenoid Cleavage Dioxygenase Genes, *OsCCD1, 4a, and 4b*, Increases Carotenoid Content of Rice. *J. Exp. Bot.* 2018, 69(21), 5105-5116.

Kusaba, M.; Miyahara, K.; Iida, S.; Fukuoka, H.; Takano, T.; Sassa, H. *et al.* Low glutelin content 1: A dominant mutation that suppresses the glutelinmultigene family via RNA silencing in rice. *Plant Cell* 2003, 15, 1455-1467.

Le, L.Q.; Lorenz, Y.; Scheurer, S.; Fotisch, K.; Enrique, E. *et al.* Design of tomato fruits with reduced allergenicity by dsRNAi-mediated inhibition of ns-LTP (Lyc e 3) expression. *Plant Biotech. J.* 2006, 4(2), 231-242

Lee, S.I.; Kim, H.U.; Lee, Y.H.; Suh, S.C.; Lim, Y.P.; Lee, H.Y.; Kim, H.I. Constitutive and seed-specific expression of a maize lysine-feedback insensitive dihydrodipicolinate synthase gene leads to increased free lysine levels in rice seeds. *Mol. Breed.* 2001, 8, 75–84.

Liu, Q.; Singh, S.P.; Green, A.G. High-stearic and High-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. *Plant Physiol.* 2002, 129(4),1732-1743.

Majumder, S.; Datta, K.; Datta, S.K. Rice Biofortification: High Iron, Zinc, and Vitamin-A to Fight against "Hidden Hunger". *Agronomy* 2019, 9(12), 803.

Mead, M. N. Nutrigenomics: the genome--food interface. *Environ. Health Perspect.* 2007, 115(12), A582-A589.

Mohanpuria, P.; Kumar, V.; Ahuja, P.S. et al. Producing low-caffeine tea through post-transcriptional silencing of caffeine synthase mRNA. *Plant Mol. Biol.* 2011, 76, 523–534.

Ogita, S.; Uefuji, H.; Morimoto, M. *et al.* Application of RNAi to confirm theobromine as the major intermediate for caffeine biosynthesis in coffee plants with potential for construction of decaffeinated varieties. *Plant Mol. Biol.* 2004, 54, 931–941.

Qin, X.; Liu, J.H.; Zhao, W.S.; Chen, X.J.; Guo, Z.J.; Peng, Y.L. Gibberellin 20-oxidase gene *OsGA20ox3* regulates plant stature and disease development in rice. *Mol. Plant Microbe Interact.* 2013, 26(2), 227-239

Regina, A.; Kosar-Hashemi, B.; Li, Z.; Rampling, L.; Cmiel, M.; Gianibelli, C. High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proc. Natl. Acad. Sci. USA,* 2006, 103, 3546–3551.

Roychoudhury, A. Agronomic and Genetic Biofortification of Rice Grains with Microelements to Assure Human Nutritional Security. *SF J. Agri. Crop Manag.* 2020, 1(1), 1005.

Saurabh, S.; Vidyarthi, A.S.; Prasad, D. RNA interference: concept to reality in crop improvement. *Planta* 2014, 239, 543–564.

Segal, G.; Song, R.; Messing, J. A new opaque variant of maize by a single dominant RNA-interference-inducing transgene. *Genetics.* 2003,165(1), 387-397.

Sestili, F.; Janni, M.; Doherty, A.; Botticella, E.; D'Ovidio, R.; Masci, S.; Jones, H.D.; Lafiandra, D. Increasing the amylose content of durum wheat through silencing of the *SBEIIa* genes. *BMC Plant Biol.* 2010, 10,144.

Sharma, P.; Aggarwal, P.; Kaur, A. Biofortification: A new approach to eradicate hidden hunger, *Food Reviews International.* 2017, 33(1), 1-21.

Shimada, T.; Otani, M.; Hamada, T.; Kim, S. Increase of amylose content of sweetpotato starch by RNA interference of the starch branching enzyme II gene (*IbSBEII*). *J. Plant Biotechnol.* 2006, 23, 85-90.

Siritunga, D.; Sayre, R. T. Generation of cyanogen-free transgenic cassava. *Planta*. 2003, 217(3), 367-373.

Sun, L.; Yuan, B.; Zhang, M.; Wang, L.; Cui, M.; Wang, Q.; Leng, P. Fruit-specific RNAi-mediated suppression of *SlNCED1* increases both lycopene and β-carotene contents in tomato fruit. *J. Exp. Bot.* 2012, 63(8), 3097-3108.

Sunilkumar, G.; Campbell, L. M.; Puckhaber, L.; Stipanovic, R..; Rathore, K. S. Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. *Proc. Natl. Acad. Sci. USA,* 2006, 103, 18054–18059.

Tang, G.; Galili, G. Using RNAi to improve plant nutritional value: from mechanism to application. *Trends Biotechnol.* 2004, 22(9), 463-469.

Teo, G.; Suziki, Y.; Uratsu, S. L.; Lampien, B.; Ormonde, N. *et al.* Silencing leaf sorbitol synthesis alters long-distance partioning and apple fruit quality. *Proc. Natl. Acad. Sci. USA,* 2006, 103, 18842–18847.

Weise, S. E.; Aung, K.; Jarou, Z. J.; Mehrshahi, P.; Li, Z.; Hardy, A. C.; Carr, D. J.; Sharkey, T. D. Engineering starch accumulation by manipulation of phosphate metabolism of starch. *Plant Biotech. J.* 2012, 10(5), 545-554.

Williams, C. L. Importance of dietary fiber in childhood. *J. Am. Diet. Assoc.* 1995, 95(10), 1140-1146.

Wilson, R. C.; Doudna, J. A. Molecular mechanisms of RNA interference. *Annu. Rev. Biophys.* 2013, 42, 217-239.

Xiong, A-S.; Yao, Q-H.; Peng, R-H.; Li, X.; Han, P-L.; Fan, H-Q. Different effects on ACC oxidase gene silencing triggered by RNA interference in transgenic tomato. *Plant Cell Rep.* 2005, 23, 639-646.

Yang, Q. Q.; Yu, W. H.; Wu, H. Y.; Zhang, C. Q.; Sun, S. S.; Liu, Q. Q. Lysine biofortification in rice by modulating feedback inhibition of aspartate kinase and dihydrodipicolinate synthase. *Plant Biotechnol. J.* 2021, 19(3), 490-501.

Yu, B.; Lydiate, D. J.; Young, L. W.; Schafer, U. A.; Hannoufa, A. Enhancing the carotenoid content of *Brassica napus* seeds by downregulating lycopene epsilon cyclase. *Transgenic Res.* 2007, 17, 573-585.

Zhang, L.; Jing, F.; Li, F.; Li, M.; Wang, Y. *et al.* Development of transgenic *Artemisia annua* (Chinese wormwood) plants with an enhanced content of artemisinin, an effective anti-malarial drug, by hairpin-RNA-mediated gene silencing. *Biotechnol. Appl. Biochem.* 2009, 52(Pt 3), 199-207.

Zhang, M. K.; Liu, F. J.; Tao, Y. J.; Hu, X.; Li, R. S.; Xu, Q. L. Cloning of *CASase* gene from *Lathyrus sativus* and construction of its RNAi vector. *Acta Agrestia Sinica*. 2016, 24, 911–914.

Zhang, Y. Y.; Li, H. X.; Shu, W. B.; Zhang, C. J.; Ye, Z. B. RNA interference of a mitochondrial APX gene improves vitamin C accumulation in tomato fruit. *Scientia Hort.* 2011, 129, 220–226.

Zhu, X.; Gong, H.; He, Q.; Zeng, Z.; Busse, J. S. *et al.* Silencing of vacuolar invertase and asparagine synthetase genes and its impact on acrylamide formation of fried potato products. *Plant Biotechnol. J.* 2016, 14(2), 709-718.