RNA INTERFERENCE: A NEW TECHNOLOGY FOR CROP IMPROVEMENT

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ABSTRACT

In mid-ninety's, the discovery of RNA interference (RNAi) added a new dimension in the regulation of gene expression by different types of RNA. It soon caught the worldwide attention and a number of reviews have been published to describe the RNAi phenomenon both in plants and animals. The technology became a powerful tool to understand the functions of individual genes and also proved useful for molecular breeders to produce improved crop varieties. This review article summarizes the historical background of RNAi, describes the mechanism of RNA induce silencing. Further, article gives applications of RNAi used to produce improved crop varieties.

KEY WORDS: RNAi, technology

INTRODUCTION

Crops plants are the backbone of all life on Earth and it is very essential resource for human well-being not just for their basic needs such as food, fodder, and shelter but also on other crop plant-derived products including gum, resin, timber, fiber, oil, dyes, pharmaceutically important secondary metabolites, drugs, and fossil fuels etc. As burgeoning world population, the demand for crops plant increases, which has lead to the future food security, malnutrition and famine (Brown and Funk, 2008; Lobell *et al*., 2008; Godfray *et al*., 2010). The quality and quantity of crops have been improved by conventional plant breeding methods, which are well known and still in the practice, but these are time consuming, laborious and the limited genetic resources of most crops have left little room for continued improvement by these means. There are many reasons for the limited genetic resources available for breeding (Hoisington *et al*., 1999). Two of the most important ones are: (i) loss of gene pools occurring during the domestication and breeding of crop plants (Lee, 1998); and (ii) many of the natural gene traits that may be beneficial in one crop plant tissue, such as seeds and fruits, may be deleterious in other crop plant tissues such as vegetative tissues (Negrutiu *et al.* 1984; Frankard *et al.*, 1992; Zhu and Galili 2003). These problems could be tackles effectively in shorter period of time with available new technologies of genomics and proteomics such as, molecular breeding and recombinant DNA (Mittler and Blumwald, 2010; Tester and Langridge, 2010). This approaches offers rapid introgression

of novel genes and traits into elite crops to raise yield, nutritional value, and confer resistance to abiotic and biotic stresses.

The potentiality to transfer and express genes from other than crops sources into edible crops has raised apprehensions about the possible dangers to human beings and the environment and also erosions of genetic diversity. Transgene movement to other varieties and wild relatives leading to monster crops, erosion of genetic diversity, and ecological disturbances are major worries. Hence, before releasing transgenic crops for regular use, transgenic crops are subjected to intricate tests to understand the risks and to ensure safety; development of transgenic crops thus needs additional time, cost and expertise (Jagtap *et al*., 2011). Therefore, there is a need to develop new strategies and safe ways for crop improvement, which could prove to be more acceptable to the general public. In this regard, RNA silencing or RNA interference (RNAi) technology has attracted the minds of researchers working in different areas of molecular biology throughout the world.

RNA interference is a novel gene regulatory mechanism that limits the transcript level by either suppressing transcription (TGS) or by activating a sequence- Specific RNA degradation process [PTGS/RNA interference (RNAi)] (Agrawal *et al*., 2003).

Discovery of RNAi

Molecular biologists had applied various methods such as insertion of TDNA elements and transposons, treatment with mutagens or irradiation and antisense RNA suppression to knockout gene expression at the mRNA level for the last few years prior to the discovery of RNAi. Apart from being time-consuming, the above methods did not always work satisfactorily. This background lead to the discovery a novel phenomenon called as RNAi.

Napoli and Jorgensen were the first to report an RNAi type of phenomenon in 1990 (Napoli *et al*., 1990). The goal of their studies was to determine whether chalcone synthase (CHS), a key enzyme in flavonoid biosynthesis, was the rate-limiting enzyme in anthocyanin biosynthesis. The anthocyanin biosynthesis pathway is responsible for the deep violet colouration in petunias. In an attempt to generate violet petunias, Napoli and Jorgensen over expressed chalcone synthase in petunias, which unexpectedly resulted in white petunias. The levels of endogenous as well as introduced CHS were 50-fold lower than in wild-type petunias, which lead them to hypothesize that the introduced transgene was "co-suppressing" the endogenous CHS gene. A similar phenomenon reported in *Neurospora crassa* noting that introduction of homologous RNA sequences caused "quelling" of the endogenous gene (Romano and Macino, 1992). This phenomenon was not limited to plants.

Guo and Kemphues injected the antisense strand RNAs of certain endogenous gene (par-1 mRNA) into worms (*Caenorhabditis elegans*), with intension that those antisense strands may hybridized to corresponding gene and in turn block translation. At that time, antisense was one of the most attractive means of eliminating gene expression. Antisense was thought to function by hybridization with endogenous mRNAs resulting in double- stranded RNA (dsRNA), which either inhibited translation or was targeted for destruction by cellular ribonucleases. Surprisingly, when Guo and colleagues performed control

experiments using only the sense par-1 RNA, which would not hybridize with the endogenous par-1 transcript, the par-1 message was still targeted for degradation. This finding caused investigators to rethink the central dogma (Guo and Kemphues, 1995). This mystery was solved in 1998, when Fire and Mello tested the synergy effect of sense and antisense strand together. They found that double-stranded RNA was at least ten-fold or perhaps a hundred fold more potent as a silence trigger than was sense or antisense alone. Furthermore, they pointed out the repression effect observed by Guo and Kemphues, was in fact caused by trace amount of double-stranded RNA contamination in those samples (Fire *et al*., 1998). Fire and Mello were awarded the Nobel Prize in Physiology or Medicine in 2006 for their discovery of RNA interference (Anonymous, 2006). Today we have a greater understanding of the components that are part of the RNAi pathway, the efficiency with which these components functions the specificity of sequence recognition and cleavage of cellular mRNA and many of the key requirements for designing and generating extremely effective RNAi reagents. RNAi analysis gives one a new powerful strategy to analyze gene function even in model organisms where molecular genetic technologies can be applied. For example, RNAi methods greatly enhance the ease of generating double or triple-loss-of-function "mutants," as creating these mutants using more traditional approaches relies on rare crossover events (Kennerdell and Carthew, 1998).

Mechanism of RNAi

Synthesis of proteins is achieved by a process called as central dogma that involves (1) Transcription of DNA into mRNA in the nucleus, (2) Transport of the mRNA strand to the ribosome (mostly rRNA), and (3) complimentary base pair binding of tRNA with an attached amino acid, whereby the mRNA strand is translated into a protein.

RNA interference, also known as post transcriptional gene silencing (PTGS) in which RNA interfere with or inhibit RNA (mRNA, rRNA, or tRNA) at post-transcription level which ultimately stalled synthesis of protein (Singh Apekshita *et al*., 2011). There are two small RNAs in the RNAi pathway: small interfering RNAs (siRNAs) and micro RNAs (miRNAs) that are generated via processing of longer dsRNA and stem loop precursors (Hammond *et al*., 2000; Bernstein *et al*., 2001a; Stevenson, 2004). Dicer enzymes play a critical role in the formation of these two effectors of RNAi (Elbashir *et al*., 2001). They can cleave long dsRNAs and stem loop precursors into siRNAs and miRNAs in an ATP-dependent manner, respectively (Angaji *et al*., 2010). The common goal of all RNAi technologies is to produce sufficient amounts of double-stranded RNA (dsRNA) within plants which has homology with endogenous messenger RNAs and can trigger the initiation of the silencing mechanism. The most efficient delivery methods for dsRNA in plants today are (1) inoculation of plants with engineered plant viruses which produce in their life cycles dsRNA intermediates (Robertson, 2004) and (2) transformation of plants with transgene constructs from which the RNA transcripts are folded into dsRNA structures (Waterhouse *et al*., 1998). Introduced double-stranded RNA can induce degradation of target RNA with sequence similarity (Fire *et al*., 1998). The double-stranded RNA is fragmented into small RNA fragments with a size of ~21

nucleotides (Bernstein *et al*., 2001b) by the RNase III-like protein Dicer (Hammond *et al*, 2000). The antisense strand or guide strand of siRNA molecule is incorporated into a nuclease containing RISC complex upon the loss of sense strand of the siRNA duplex by an RNA helicase activity (Kusaba, 2004). RISC with antisense siRNA sequence then targets the homologous transcript by base-pairing interaction and argonaute is an endonuclease which cleaves the mRNA or blocks the translation leading to inhibition of protein synthesis (Bartel, 2004) (Fig. 1). The degree of degradation of the targeted plant RNAs can vary from partial to complete degradation and depends on exogenous factors, e.g. temperature as well as endogenous factors, e.g. the physiological status of the plant (Metzlaff, 2005).

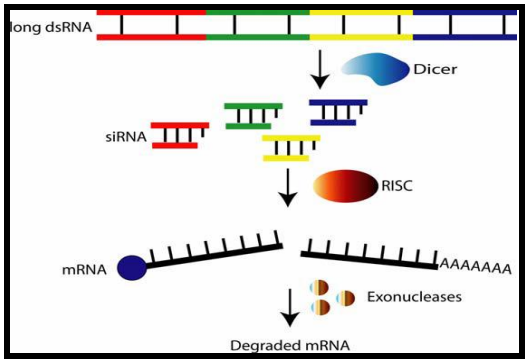

Fig. 1: RNAi mechanism in which dicer cuts Long dsRNA is cut into smaller fragments, called siRNA. The siRNAs are then incorporated into RISC. The siRNA-RISC complex then targets a sequence, complementary to the siRNA, in a piece of mRNA. The mRNA is cut by RISC exposing it to cellular endonucleases that eventually degrade the mRNA.

Application of RNAi for improvement of crop

RNAi technology may be used for such applications as gene silencing thereby generating improved crop varieties in terms of disease-insect resistance, enhancing nutritional qualities, and much more (Table 1).

TERMINOLOGY

Gene Knockout :- It is a genetic technique in which one of an organism's genes is inactive or inactive expression of gene to study a function of loss gene.

Dicer:- Dicer is an endoribonuclease in the RNase III family that cleaves double-stranded RNA (dsRNA) and pre-microRNA (miRNA) into short double-stranded RNA fragments called small interfering RNA (siRNA) about 20-25 nucleotides long

Argonaute: - Argonaute proteins are the catalytic components of the RNA-induced silencing complex (RISC), the protein complex responsible for the gene silencing

RISC: - RNA-induced silencing complex, or RISC, is a multiprotein complex that incorporates one strand of a small interfering RNA (siRNA) or micro RNA (miRNA) which activates RNase and cleaves the RNA.

Transgenic crop: - it has a novel combination of genetic material obtained through the use of modern biotechnology. Transgenic indicates that a transfer of genes has occurred using recombinant DNA technology

Down-regulation: - decrease activity or expression of gene in cell.

RNA helicase: - exhibit helicase activity which separating two annealed RNA strands.

CONCLUSION

In coming 30 years the demand for food for increasing population will be two billion stated by the FAO (Food and Agriculture Organization), to meet this herculean task we needs more and more molecular tools to reduce current crop loss and feed extra mouths. The RNAi technology, described in this article, describes one such powerful

innovation. If judiciously used, this technology may go a long way to narrow the gap through production of disease-, insect- and virus resistant, nutritionally rich and toxic-free crops. One of the major purposes of the present review article is to help policy makers in food deficient countries to understand how scientific breakthroughs such as RNAi technology may be helpful in tackling this gigantic problem of feeding an additional 2 billion people over the next 30 years from an increasingly fragile natural resource base. However, any new technology involving the gene manipulation may be opposed by anti-GM groups severely limiting its effectiveness or wider use. Since this technology offers a great potential in understanding gene functions and utilize them to improve crop quality and production, it is a matter of time before we see the products of this RNAi research in the farmers' fields around the world.

REFERENCES

Agrawal Neema, Dasaradhi, P. V. N., Mohmmed Asif, Malhotra Pawan, Bhatnagar, R. K. and Mukherjee, S. K. (2003). RNA Interference: Biology, Mechanism, and Applications *Micro. Mol. Bio. Rev*., 67(4): 657–685.

Ali, N., Paul, S., Gayen, D., Sarkar, S. N. and Datta, S. K. (2013b). Development of low phytate rice by RNAi mediated seed-specific silencing of Inositol 1,3,4,5,6-Pentakisphosphate 2-Kinase gene (IPK1). *PLoS ONE* 8(7): e68161. doi:10.1371/journal.pone.0068161.

Ali, N., Paul, S., Gayen, D., Sarkar, S. N., Datta, S. K and Datta, K..(2013a). RNAi mediated down regulation of myo-inositol-3-phosphate synthase to generate low phytate rice. *Rice*, **6**: 12 doi:10.1186/1939-8433-6-12.

Altenbach, S. B., Allen P. V (2011). Transformation of the US bread wheat 'Butte 86' and silencing of omega-5 gliadin genes, *GM Crops*, 2: 66-73.

Angaji, S. A., Hedayati, S. S., Hosein poor, R., Samad poor, S., Shiravi, S. and Madani, S. (2010). Application of RNA interference in plants, *Plant Omics J*., **3**(3): 77-84.

Anonymous (2006). "The Nobel Prize in Physiology or Medicine 2006". Nobelprize.org.http://www.nobelprize.org/nobel_prizes/medicine/laureates/2006/

Anonymous (2007). "Studies force new view on biology of flavonoids", by David Stauth, EurekAlert!. Adapted from a news release issued by Oregon State University. URL accessed.

Augustine, R., Mukhopadhyay, A. and Bisht, N. C. (2013). Targeted silencing of BjMYB28 transcription factor gene directs development of low glucosinolate lines in oilseed *Brassica juncea. Plant Biotechnol J.*, 11(7): 855-866.

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism and function. *Cell*, **116**: 281–297.

Bernard, A. (2008). Cadmium & its adverse effects on human health, *Indian J. Med. Res*., **128**: 557-564.

Bernstein, E., Caudy, A. A., Hammond, S. M. and

Hannon, G. J. (2001a). Role for a bidentate ribonuclease in the initiation step RNA interference. *Nature*, **409**: 363–366.

Bernstein, E., Caudy, A. A., Hammond, S. M. and Hannon, G. J. (2001b): Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*, **409**: 295-296.

Bhinu, V. S., Schafer, U. A., Li, R., Huang, J. and Hannoufa, A. (2009). Targeted modulation of sinapine biosynthesis pathway for seed quality improvement in *Brassica napus*. *Transgenic Res.*, **18**: 31–44.

Brian, M. W., Cristobal, U., Jorge, D. and Michael A. G. (2009). Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *J. Exp. Bot*.,.**60**: 4263-4274.

Brown, M. E. and Funk, C. C. (2008) Climate: food security under climate change. *Sci.*, **319**:580–581.

Chew, B. L. and Seymour, G. B. (2013). The effects of glutamate decarboxylase (GAD) RNAi knockout in tissue cultured transgenic tomato (*Solanum lycopersicum*). *POJ*, **6**(1): 13-17.

Civitelli, R., Villareal, D. T., Agnusdei, D., Nardi, P, Avioli, L. V. and Gennari, C. (1992). Dietary L-lysine and calcium metabolism in humans. *Nutrition*, **8**(6):400-405.

Coetzer, C., Corsini, D., Love, S., Pavek, J. and Tumer, N. (2001). Control of enzymatic browning in potato (*Solanum tuberosum* L.) by sense and antisense RNA from tomato polyphenol oxidase. *J. Agric. Food Chem.*, **49**(2):652-657.

Davuluri, G. R., van Tuinen, A., Fraser, P. D., Manfredonia, A., Newman, R., Burgess, D., Brummell, D. A., King, S. R., Palys, J., Uhlig, J., Bramley, P. M., Pennings, H. M. and Bowler, C. (2005). Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. *Nature Biotechno*., **23**: 890–895.

Elbashir, S. M., Martinez, J., Patkaniowska, A., Lendeckel, W. and Tuschl, T. (2001). Functional anatomy of siRNAs for mediating efficient RNAi in *Drosophila melanogaster* embryo lysate. *EMBO J.*, **20**:6877-6888.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, **391**: 806-811.

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

Transgenic Res., **17**(5):839-850.

Fornale, S., Capellades, M., Encina, A., Wang, K., Irar, S., Lapierre, C., Katia, Ruel, J. J., Berenguer, J., Puigdomènech, P., Rigau, J. and Caparrós-Ruiz, D. (2012). Altered lignin biosynthesis improves cellulosic bioethanol production in transgenic maize plants down-regulated for cinnamyl alcohol dehydrogenase, *Mol. Plant*, **5**: 817-830.

Francesco, S., Michela, J., Angela, D., Ermelinda, B., Renato, D., Stefania, M., Huw, D. J. and Domenico, L. (2010). Increasing the amylose content of durum wheat through silencing of the SBEIIa genes, *BMC Plant Biology*, **10**:144. doi:10.1186/1471-2229-10-144.

Frankard, V., Ghislain, M., & Jacobs, M. (1992). Two feedbacks in sensitive enzymes of the aspartate pathway in *Nicotiana sylvestris*. *Plant Physio*., **99**: 1285–1293.

Gil-Humanes, J., Pistón, F., Hernando, A., Alvarez, J. B., Shewry, P. R. and Barro, F. (2008)..Silencing of γ-gliadins by RNA interference (RNAi) in bread wheat. *J. Cereal Sci*., **48**: 565-568.

Gil-Humanes, J., Pistón, F., Tollefsen, S., Sollid, L. M. and Barro, F. (2010). Effective shutdown in the expression of celiac disease-related wheat gliadin T-cel epitopes by RNA interference. *PNAS*, **107**(39): 17023-17028.

Gilissen, L. J. W. J., Bolhaar, S. T. H., Matos, C. I., Rouwendal, G. J. A., Boone, M. J., Krens, F. A., Zuidmeer, L., Van Leeuwen, A., Akkerdass, J., Hoffmann-Sommergruber, K., Knulst, A. C., Bosch, D., Van De Weg, W. E. and Van Ree, R. (2005). Silencing of the major apple allergen Mal 1 by using RNA interference approach. *J. Allergy Clinic. Immuno.*, **115**: 369-384.

Godfray, H. C. J., Beddington, J.R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. Z., Pretty, J., Robinson, S., Thomas, S. M. and Toulmin, C. (2010). Food security: the challenge of feeding 9 billion people. *Sci*., **327**: 812–818.

Grootboom, A. W. (2010). A thesis on effect of RNAi down-regulation of three lysine-deficient kafirins on the seed lysine content of sorghum [*Sorghum bicolor* (L.) Moench], PhD in Plant Science submitted to University of Pretoria, Pretoria, Gauteng, South Africa.

Guan, S., Ma, Y., Liu, H., Liu, S., Liu, G., Zhao, L. and Wang, P. (2012). Increasing the amylose content of maize through silencing of sbe2a genes, *African J. Biotechno*., **11**(30): 7628-7636.

Guo, S. and Kemphues, K. J. (1995). par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically

distributed. *Cell*, **81**: 611–620.

Gupta, A., Pal, R. K. and Rajam, M. V. (2013). Delayed ripening and improved fruit processing quality in tomato by RNAi-mediated silencing of three homologs of 1-aminopropane-1-carboxylate synthase gene. *J Plant Physiol*., **170**(11): 987-995.

Hammond, S. M., Bernstein, E., Beach, D. and Hannon, G. J. (2000). An RNA directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *Nature*, **404**(6775): 293-296.

Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J. M., Skovmand, B., Taba, S., and Warburton, M. (1999). Plant genetic resources: What can they contribute toward increased crop productivity? *Proceedings of the National Academy of Sciences of the USA*, **96**: 5937 -5943.

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

Jagtap, U. B., Gurav, R. G. and Bapat, V. A. (2011). Role of RNA interference in plant improvement. *Naturwissenschaften*, **98**(6): 473 - 492.

Jiang, H., Zhang, J., Wang, J., Xia, M., Zhu, S. and Cheng, B. (2013). RNA interference-mediated silencing of the starch branching enzyme gene improves amylose content in rice. *Genet. Mol. Res.*, 12(3); doi: 10.4238/2013.

Julie, B., Aurélia, B., Christine, G., Catherine, R., Caroline, T., Pierre, B., Mohamed, F. B. and Said, M. .(2012). Down-regulation of the TaGW2 gene by RNA interference results in decreased grain size and weight in wheat. *J. Exp. Bot*., **63**: 5945-5955.

Karlova, R., Rosin, F. M., Busscher-Lange, J., Violeta Parapunova Phuc T. Do, Fernie, A. R., c Paul, D. F., d Charles Baxter, e Gerco C. Angenent and Ruud A. deMaagd. (2011). Transcriptome and Metabolite Profiling Show That APETALA2a Is a Major Regulator of Tomato Fruit Ripening, *The Plant Cell*, **23**: 923–941.

Kennerdell, J. R. and Carthew, R. W. (1998). Use of dsRNA mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell*., **95**(7): 1017-26.

Krath, B. N., Eriksen, F. D., Pedersen, B. H., Gilissen, L. J. W. J., Van De Weg, W. E. and Dragsted, L. O. (2009). Development of hypo-allergenic apples: silencing of the major allergen Mal d 1 gene in 'Elstar' apple and the effect of grafting, *J. Horti. Sci. Biotechno.,* 84: 52–57.

Kumar, T., Dweikat, I., Sato, S., Ge, Z., Nersesian, N., Chen, H., Elthon, T., Bean, S., Ioerger, B. P., Tilley, M. and Clemente, T. (2012).

Modulation of kernel storage proteins in grain sorghum (*Sorghum bicolor* (L.) Moench), Plant Biotechno. J., 10: 533–544.

Kusaba, M. (2004). RNA interference in crop plants. *Current Opinions in Biotechno*., **15**:139-143.

Kusaba, M., Miyahara, K., Lida, S., Fukuoka, H., Takario, T., Sassa, H., Nishimura, M. and Nishio, T. (2003). Low glutenin content 1: a dominant mutation that suppresses the glutenin multigene family via RNA silencing in rice. *Plant Cell*, **15**: 1455-1467.

Le, L. Q., Mahler, V., Lorenz, Y., Scheurer, S., Biemelt, S., Vieths, S. and Sonnewald, U. (2006) Reduced allergenicity of tomato fruits harvested from Lyc e 1-silenced transgenic tomato plants. *J. Allergy Clin. Immunol.*, **118**(5): 1176-1183.

Lee, M. (1998). Genome projects and gene pools: New germplasm for plant breeding? *Proceedings of the National Academy of Sciences of the USA*, **95**: 2001–2004.

Li, J. C., Guo, J. B. Xu, W. Z. and Ma, M. (2007). RNA interference-mediated silencing of phytochelatin synthase gene reduce cadmium accumulation in rice seeds. *J. Integr. Plant Biol*., **49**(7): 1032–1037.

Liu, Q., Singh, S. P. and Green, A. G. (2002). High-stearic and high-oleic cotton seed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. *Plant Physio.*, **129**: 1732–1743.

Lobell, D. B., Burke, M. B., Tebaldi, C., Mastrandrea, M. D., Falcon, W. P. and Naylor, R. L. (2008). Prioritizing climate change adaptation needs for food security in 2030. *Sci*., **319**: 607–610.

Mary, J. G., Ricardo, J. S. and Brian, M. W. (2013). Nutrient partitioning and grain yield of TaNAM-RNAi wheat under abiotic stress. *Plant soil*, 371: 573-591.

Melendez-Martínez, A. J., Vicario, I. M. and Heredia, F. J. (2004). Nutritional importance of carotenoid pigments. Arch Latinoam Nutr., **54**(2):149-154.

Metzlaff, M. (2005), Applications of RNAi in crop improvement, *Pflanzenschutz-Nachrichten Bayer*, **58**(1): 51-59.

Mittler, R. and Blumwald, E. (2010). Genetic engineering for modern agriculture: challenges and perspectives. *Ann Rev Plant Biol*., **61**: 443–462.

Napoli, C., Lemieux, C. and Jorgensen, R. (1990). Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. Pl. Cell, 2: 279–289.

Negrutiu, I., Cattoir-Reynearts, A., Verbruggen, I. and Jacobs, M. (1984). Lysine overproducer mutants with an altered dihydrodipicolinate synthase from protoplast culture of *Nicotiana sylvestris* (Spegazzini and Comes). *Theor. Appl. Genet*., **68**: 11–20.

Niu, X., Tang, W., Huang, W., Ren, G., Wang, Q., Luo, D., Xiao, Y., Yang, S., Wang, F., Lu, B., Gao, F., Lu, T. and Liu, Y. (2008). RNAi-directed down regulation of OsBADH2 results in aroma (2-acetyl-1-pyrroline) production in rice (*Oryza sativa* L.), *BMC Plant Bio.*, **8**:100 doi:10.1186/1471-2229-8-100.

Ogita, S., Uefuji, H., Yamaguchi, Y., Koizumi, N. and Sano, H. (2003). Producing decaffeinated coffee plants. *Nature*, **423**: 823.

Otani, M., Hamada, T., Katayama, K., Kitahara, K., Kim, S. H., Takahata, Y., Suganuma, T. and Shimada, T. (2007). Inhibition of the gene expression for granule-bound starchsynthase I by RNA interference in sweet potato plants. *Plant Cell Reports*, **26**: 1801–1807.

Pearson, A.W., Butler, E.J. and Fenwick, R.G. (1980). Rapeseed meal and egg taint: the role of sinapine. J. *Sci. Food Agric.*, **31**: 898–904.

Peters, S., Imani, J., Mahler, V., Foetisch, K., Kaul, S., Paulus, K. E., Scheurer, S., Vieths, S. and Kogel, K. H. (2011). Dau c 1.01 and Dau c 1.02-silenced transgenic carrot plants show reduced allergenicity to patients with carrot allergy. *Transgenic Res.*, **20**(3): 547-556.

Ral, J. P., Bowerman, A. F., Li, Z., Sirault, X., Furbank, R., Pritchard, J. R., Bloemsma, M., Cavanagh, C. R., Howitt, C. A. and Morell, M. K. (2012). Down-regulation of Glucan, Water-Dikinase activity in wheat endosperm increases vegetative biomass and yield. *Plant Biotechnol. J.*, **10**(7): 871-882.

Rathore, K. S., Sundaram, S., Sunilkumar, G., Campbell, L. M., Puckhaber, L., Marcel, S., Palle, S. R., Stipanovic, R. D. and Wedegaertner, T. C. (2011). Ultra-low gossypol cottonseed: generational stability of the seed-specific, RNAi-mediated phenotype and resumption of terpenoid profile following seed germination. *Plant Biotechno. J.*, 10(2): 174-183.

Regina, A., Bird, A., Topping, D., Bowden, S., Freeman, J., Barsby, T., Kosar-Hashemi, B., Li, Z., Rahman, S. and Morell, M. (2006). High-amylose wheat generated by RNA interference improves indices of large-bowel health in rats. *Proceedings of the National Academy of Science USA*, **103**: 3546–3551.

Regina, A., Kosar-Hashemi, B., Ling, S., Li, Z., Rahman, S. and Morell, M. (2010). Control of starch branching in barley defined through differential RNAi suppression of starch branching enzyme IIa and IIb. *J. Exptl. Bot.*, **61**(5): 1469–1482.

Robertson, D. (2004). VIGS vectors for gene silencing: many targets, many tools. *Annual Rev. Plant Biol.*, **55**, 495-591.

Romano, N. and Macino, G. (1992) Quelling: transient

inactivation of gene expression in *Neurospora crassa* by transformation with homologous sequences. *Mol. Microbiol.,* 6: 3343–3353.

Schijlen, E. G., de Vos, W. M., Martens, C. H. R., Martens, S., Jonker, H. H., Rosin, F. M., Molthoff, J. W., Tikunov, Y. M., Angenent, G. C., van Tunen, A. J. and Bovy, A. G. (2007). RNA interference silencing of chalcone synthase, the first step in the flavonoid biosynthesis pathway, leads to parthenocarpic tomato fruits. *Pl. Physiol*., **144**: 1520 -1530.

Segal, G., Song, R. and Messing, J. (2003). A New Opaque Variant of Maize by a Single Dominant RNA-Interference-Inducing Transgene. *Genet*., **165**: 387–397.

Shimada, T., Otani, M., Hamada, T. and Kim, S.H. (2006). Increase of amylose content of sweet potato starch by RNA interference of the starch branching enzyme II gene (IbSBEII). *Plant Biotechno*., **23**: 85–89.

Singh Apekshita, Kumar Benu and Srivastava A. K. (2011). Metabolic engineering of crops using RNA interference. *AsPac J. Mol. Biol. Biotechnol*., **19**(4): 137-148.

Song, Y. H. (2009). A thesis on the silencing of polyphenol oxidase genes in potato triggered by RNA interference. Master of Genetics in Agricultural Science, China.

Stevenson, M. (2004). Therapeutic Potential of RNA Interference. *The New England Journal of Medicine,* **351**:1772-1777.

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

Sunilkumar, G., Campbell, L. M., Puckhaber, L., Stipanovic, R. D., and Rathore, K. S. (2006). Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol, *PNAS*, **103**: 18054-18059.

Tang, G., Galili, G. and Zhuang, X. (2007). RNAi and microRNA: breakthrough technologies for the improvement of plant nutritional value and metabolic engineering. *Metabolomics*, **3**:357–369.

Tester, M. and Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Sci*., **327**: 818–822.

Tian, B., Sun, D., Lian, Y., Shu, H., Ling, H., Zang, X., Wang, B. and Pei, Z. (2011). Analysis of the RNAi targeting FAD2 gene on oleic acid composition in transgenic plants of Brassica napus, *African J. Microbiol. Res*., **5**(7): 817-822.

Uraguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida, A., Kyozuka, J., Ishikawa, S. and Fujiwara, T. (2011).

Low-affinity cation transporter (OsLCT1) regulates cadmium transport into rice grains. *PNAS*, **108**: 20959-20964.

Van Eck, J., Conlin, B., Garvin, D. F., Mason, H., Navarre, D. A. and Brown, C. R. (2007) Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene. *Amer. J. Potato Res.*, **84**: 331-342.

Wang, S., Liu, J., Feng, Y., Niu, X., Giovannoni, J. and Liu, Y. (2008). Altered plastid levels and potential for improved fruit nutrient content by down regulation of the tomato DDB1-interacting protein CUL4. *Plant J.*, **55**(1):89-103.

Waterhouse, P. M., Graham, M. W. and Wang, M. B. (1998). Virus resistance and gene silencing can be induce by simultaneous expression of sense and antisense RNA. *Proc. Natl. Acad. Sci. USA*, **95**: 13959-13964.

Wei, S., Li, X., Gruber, M.Y., Li, R., Zhou, R., Zebarjadi, A. and Hannoufa, A. (2009). RNAi-mediated suppression of DET1 alters the levels of carotenoids and sinapate esters in seeds of *Brassica napus*. *J. Agric. Food Chem.*, **57**: 5326–5333.

Wu, Y. and Messing, J. (2012). RNA Interference Can Rebalance the Nitrogen Sink of Maize Seeds without Losing Hard Endosperm. *PLoS ONE* 7(2): e32850. doi:10.1371/journal.pone.0032850.

Wu, Y., Wang, W. and Messing, J. (2012). Balancing of sulfur storage in maize seed. *BMC Plant Bio.*, **12**:77 doi: 10.1186/1471-2229-12-77.

Xia, G. (2013). Repression of Lignin Synthesis in Rice by C4H and 4CL Using RNAi. *International J. Biosci. Biochem. Bioinform.*, 3(3):

Yongsheng, L., Sherry, R., Zhibiao, Y., Cornelius, B., Ageeth van, T., Julia, V., Chris, B. and Jim, G. (2004). Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato, *PNAS*, **101**(26) ; 9897–9902.

Yuanyuan, F., Yingnan, L. I., Pei, H., Jingrong, Z., Songhu, W. and Yongsheng, L. (2012). Construction and Genetic Transformation of Tomato ARF4 RNA Interference Expression Vector with Fruit Specific Promoter. *Chinese J. Appl. Environmental Bio.*, 18(2): 206-211.

Zanor, M. I., Sonia, O., Adriano, Nunes-Nesi, Fernando, C., Marc, L., Björn, U., Christin, K., Wilfrid, B., Patrick, G., Lothar, W., Ronan, S., Yan-Hong, Z., and Alisdair, R. F. (2009). RNA Interference of LIN5 in tomato confirms its role in controlling brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. *Plant Physiol.*, **150**(3): 1204–1218.

Zhang Yu-Man, Yan Yong-Sheng, Wang Li Na, Yang Kun,

Xiao Na, Liu Yun-Feng, Fu Ya-Ping, Sun Zong-Xiu, Fang Rong-Xiang and Fang Xiao-Ying Chen (2012). A novel rice gene, NRR responds to macronutrient deficiency and regulates root growth. *Mol. Plant*, **5**: 63-72.

Zhu, X. and Galili, G. (2003). Increased lysine synthesis coupled with a knockout of its catabolism synergistically boosts lysine content and also transregulates the metabolism of other amino acids in *Arabidopsis* seeds. *Plant Cell*, **15**: 845–853.

Table 1: List of applications of RNAi in crop improvement.

Crop	Case study	Target Gene	Application	References
Rice	A variety of rice with low glutelin content 1 (LGC-1)	Lgc-1	Low glutenin content was a relief to the kidney patients unable to digest glutenin. The trait was stable and was transmitted for a number of generations. They showed that the procedure may apply to both monogenic and polygenic agronomic characters.	Kusaba *et al*., 2003
	Reduce Cadmium Accumulation in Rice Seeds	Phytochelatin Synthase Gene OsPCS1	Cadmium (Cd), one of the most toxic heavy metals, causes several health problems even at trace levels. Cd accumulation was reduced by about half in the seeds of RNAi rice. This result suggests that this new approach can be used to control heavy metal accumulation in crops.	Li *et al*., 2007
	Modulate the rice root architecture with the availability of macronutrients.	NRR (NRRa or NRRb)	Knock-down of expression of *NRRa* or*NRRb* by RNA interference resulted in enhanced rice root growth. By contrast, These results revealed that both *NRRa* and*NRRb* played negative regulatory roles in rice root growth. Findings suggest that *NRRa* and *NRRb*, acting as the key components, modulate the rice root architecture with the availability of macronutrients.	Zhang *et al*., 2012
	Generate "low-Cd rice"	OsLCT1	Accumulation of Cadmium (Cd) in rice (*Oryza sativa* L.) grains poses a potential health problem (toxic to kidney, bone demineralization, bone damage, lung cancer) (Bernard, 2008), especially in Asia. Most Cd in rice grains accumulates through phloem transport; RNAi-mediated knockdown of OsLCT1 did not affect xylem-mediated Cd transport but reduced phloem-mediated Cd transport. The regulation of OsLCT1 enables the generation of "low-Cd rice"	Uraguchi *et al*., 2011

			without negative effects on agronomical traits.	
	Repression of Lignin Synthesis	Cinnamate-4-hydroxylase (C4H) and 4-hydroxycinna-mate CoA ligase (4CL)	Inhibit key enzymes in lignin synthesis in the rice plant straw, so as to relatively decrease lignin content in rice plant straw, increase cellulose content, and eventually provide high-quality raw materials for rice straw bioavailability.	Xia, 2013
	Improves amylose content	RBE3	Transgenic rice amylose content had an average increase of 140%. The highest rice amylose content was 47.61% and the growth rate increased 238% compared to the non-transgenic controls. Furthermore, the RBE3 gene is negatively correlated with spike size in rice. This transgenic rice is important material for amylose content production in industry.	Jiang *et al*., 2013
	Aroma (2-acetyl-1-pyrroline) production	OsBADH2	Aromatic rice is popular worldwide because of its characteristic fragrance. altered expression levels of OsBADH2 gene influence aroma accumulation.	Niu *et al*., 2008
	Generate low phytate rice	myo-inositol-3-phosphate synthase	The lower phytate level has led to an increment of divalent cations, of which a 1.6 fold increase in the iron concentration in milled rice seeds was noteworthy. This increase could be attributed to reduced chelation of divalent metal (iron) cations, which may correlate to higher iron bioavailability in the endosperm of rice grains.	Ali[a] *et al*., 2013
		Inositol 1,3,4,5,6-Pentakisphosphate 2-Kinase Gene (IPK1)	The low-phytate rice seeds also accumulated 1.8-fold more iron in the endosperm due to the decreased phytic acid levels.	Ali[b] *et al*., 2013

Wheat	Transgenic wheat with increased levels of amylase content	Starch-branching enzyme (SBE) II (SBEIIa and SBEIIb)	This resulted in increased grain amylose content to over 70% of the total starch content (Tang *et al*., 2007). That this high-amylose wheat has a significant potential to improve human health (Protection from several disease like Colon cancer, diabetes, obesity, osteoporosis & Cardiovascular Disease) through its resistant starch content.	Regina *et al*., 2006 and
		SBEIIa		Francesco *et al*., 2010
	Regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain in wheat (*Triticum aestivum*)	NAM-B1	Major effect of the NAM genes is an increased efflux of nutrients from the vegetative tissues and a higher partitioning of nutrients to grain in wheat (*Triticum aestivum*).	Brian *et al*., 2009
	Potential nutritional benefits for Gluten Intolerant Consumers	γ-gliadins	The major protein fraction of wheat grain is gluten which is largely responsible for the functional properties of dough. Gliadins contribute mainly to the extensibility and viscosity of gluten and dough, with the polymeric glutenins being responsible for elasticity therefore to silence the expression of specific γ-gliadins that feasibility of systematically silencing specific groups of gluten proteins which is beneficial for Gluten Intolerant consumers.	Gil-Humanes, 2008
	Improved tolerance to post-anthesis abiotic stress	TaNAM genes	Delayed senescence and decreased grain protein, iron, and zinc concentrations. Resulted in improved tolerance to post-anthesis abiotic stress, and determined the effects of post-anthesis abiotic stress on N and mineral remobilization and partitioning to grain.	Mary *et al*., 2013
	Regulation of grain and weight	TaGW2	TaGW2 gene by RNA interference results in decreased grain size and weight in wheat. Therefore, these results showed that TaGW2 acts in the regulation of grain size and may provide an important tool for	Julie *et al*., 2012

			enhancement of grain yield.	
	A transgenic wheat line which have low level toxicity for Celiac Disease suffers	Gliadins	Celiac Disease (CD) is an enteropathy triggered by the ingestion of gluten proteins from wheat and the only available treatment for the disease is a lifelong gluten-exclusion diet. By down-regulation of gliadins in transgenic wheat lines which decrease levels of toxicity for CD patients.	Gil-Humanes, 2010
	A variety of bread wheat 'Butte 86' was good for immunogenic potential.	omega-5 gliadin	Down Regulation of genes encoding omega gliadins that trigger the food allergy wheat-dependent exercise-induced anaphylaxis (WDEIA) which should accelerate future research on flour quality and immunogenic potential.	Altenbach and Allen, 2011
	Increases vegetative biomass and yield.	Glucan, Water-Dikinase activity	GWD down-regulation resulted in a grain yield increase of 26% and also increased level of α-amylase activity present in the aleurone layer during grain maturation. These findings provide a potentially important novel mechanism to increase biomass and grain yield in crop improvement programmes.	Ral *et al*., 2012
Maize	high-lysine maize	22-kD alpha – zeins storage protein	It is an essential amino acid, which means that the human body cannot synthesize it. Some studies have found that lysine may be beneficial for those with herpes simplex infections. Lysine helps the body absorb calcium. Calcium is crucial for bone health thus suggesting a potential usefulness of L-lysine supplements for both preventive and therapeutic interventions in osteoporosis.(Civitelli *et al*.,1992)	Segal *et al*., 2003
	Lysine accumulation in endosperm of maize seeds	Lysine-ketoglutarate reductase Saccharopine dehydro-genase (ZLKR/SDH).		Houmard *et al*., 2007
	Balancing of sulfur storage in maize seed	Zeins storage protein	A balanced composition of amino acids in seed flour is critical because of the demand on essential amino acids for nutrition. However, seed proteins in cereals like maize, the crop with the highest yield, are	Wu *et al*., 2012

			low in lysine, tryptophan, and methionine. Therefore, understanding the mechanism of methionine accumulation in the seed could be a basis for breeding cultivars with superior nutritional quality through RNA interference (RNAi).	
	Improves Cellulosic Bioethanol Production in Transgenic Maize	Cinnamyl alcohol dehydro-genase (CAD)	Cinnamyl alcohol dehydrogenase (CAD) is a key enzyme involved in the last step of monolignol biosynthesis. By down-regulation of CAD in maize improve biosynthesis of Cellulosic bioethanol production and alter lignin synthesis which improves the nutritional and energetic values of maize.	Fornale *et al*., 2012
	Increse level of non-Zein protein	22- and 19-kDa α-zeins in Illinois High Protein (IHP)	The α-zeins were dramatically reduced, but the high total seed protein level remained unchanged by complementary increase of non-zein proteins. Moreover, the residual zein levels still allowed for a vitreous hard seed. Such dramatic rebalancing of the nitrogen sink could have a major impact in world food supply.(Wu and Messing. 2012)	Wu and Messing, 2012
	Increasing the amylose content	sbe2a	The SBE activity was significantly less than that of wild type maize, and was at most reduced by 77.9%; the amylose content was at most increased by 87.8%	Guan *et al*., 2012
Tomato	Enhanced carotenoid and flavonoid content	DE-ETHIOLATED1 (DET1)	Dietary source of carotenoids and flavonoids which are highly beneficial for human health. However, recent studies have shown the antioxidant properties of these compounds and their efficiency in the prevention of certain human diseases, such as atherosclerosis or cancer (Melendez-Martínez *et al*., 2004; Anonymous, 2007).	Davuluri *et al*., 2005
	Increases lycopene and b-carotene contents in tomato Fruit	SlNCED1		Sun *et al*., 2012
	Parthenocarpic fruits in tomato	CHS (chalcone synthase)	Parthenocarpy, the formation of seedless fruits in the absence of functional fertilization, is a	Schijlen *et al*., 2007

			desirable trait for several important crop plants, including tomato (*Solanum lycopersicum*). Seedless fruits can be of great value for consumers, the processing industry, and breeding companies. Parthenocarpy which obtain parthenocarpic tomatoes by down regulation of the flavonoid biosynthesis pathway.	
	Improved fruit nutrient quality in tomato	DDB1-interacting protein CUL4.	CUL4-RNAi repression lines provide insight regarding CUL4 function during tomato development, and revealed that this tomato cullin is important in the regulation of plastid number and pigmentation, which in turn have a direct impact on fruit nutrient quality.	Wang *et al*., 2008
		ARF4	The content of chlorophyll in green mature fruit, the weight per fruit and pericarp thickness of the transgenic lines which could improve tomato fruit quality.	Yuanyuan *et al*., 2012
	Light signaling represents genetic tools for manipulation of fruit color and nutritional value in tomato.	LeHY5 and LeCOP1LIKE	LeHY5 and LeCOP1LIKE are positive and negative regulators of fruit pigmentation, respectively. Down-regulated LeHY5 plants exhibit defects in light responses, including inhibited seedling photomorphogenesis, loss of thylakoid organization, and reduced carotenoid accumulation. In contrast, repression of LeCOP1LIKE expression results in plants with exaggerated photomorphogenesis, dark green leaves, and elevated fruit carotenoid levels.this result represent genetic tools formanipulation of fruit quality and nutritional value.	Yongsheng *et al*., 2004
	Delayed ripening and improved fruit processing quality	Three homologs of 1-aminopropane-1-carboxylate synthase	Down-regulation of ACS homologs using RNAi can be an effective approach for obtaining delayed ripening with longer shelf life and an enhanced processing quality of tomato fruits. These observations would be useful in better	Gupta *et al*., 2013

		gene	understanding of the ethylene and PA signaling during fruit ripening and molecular mechanisms underlying the interaction of these two molecules in affecting fruit quality traits.	
	Regulator of tomato fruit ripening	APETALA 2a (AP2a)	Iterations in fruit shape, orange ripe fruits, and altered carotenoid accumulation	Karlova *et al*., 2011
	Controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility	LIN5	These results are discussed in the context of current understanding of the role of sugar during the development of tomato fruit, with particular focus given to its impact on hormone levels and organ morphology.	Zanor *et al*., 2009
	Generate profiling reduced hypoallergenic tomato fruits	Lyc e 1(Lyc e 1.01 and Lyc e 1.02)	Profiling is a small actin-binding protein that contributes to the allergenic potency of many fruits and vegetables, including tomato. 2 Generate profiling reduced hypoallergenic tomato fruit s by silencing of both genes in transgenic tomato plants.	Le *et al*., 2006
	Effects of glutamate decarboxylase (GAD)	GAD	Silencing of the glutamate decarboxylase (GAD) gene would be predicted to enhance glutamate levels at the expense of GABA, this leads to altered levels of γ-aminobutyric acid (GABA) and other amino acids known to be essential for plant survival.	Chew and Seymour, 2013
Potato and Sweet potato	Amylose-free transgenic sweet potato	Granule-bound starch synthase I (GBSSI)	Granule-bound starch synthase I (GBSSI) is one of the key enzymes catalyzing the formation of amylose, a linear alpha (1, 4) D-glucan polymer, from ADP-glucose. Amylose-free transgenic sweet potato plants were produced by inhibiting sweet potato GBSSI gene expression through RNA interference.	Otani *et al*., 2007
	Higher amylose content starch	Starch branching	Transgenic sweet potato plants that produced a starch with higher	Shimada *et al*., 2006

	in sweet potato	enzyme II gene (IbSBEII)	amylose content by exploiting an RNAi technique to target the starch branching enzyme II (SBEII).	
	Enhancing beta-carotene content in potato	β-carotene hydroxylase gene (bch)	Enhanced β-carotene content by silencing of the β-carotene hydroxylase gene that converts β-carotene to the less useful zeaxanthin. However, the β-carotene to retinol conversion efficiency (21 µg of β-carotene per 1 µg retinol) proposed for developing countries suggests that a combination of strategies will be necessary to sufficiently improve the provitamin A content of potato for populations at risk of vitamin A deficiency.	Van Eck *et al*., 2007
	Silencing of browning effect in potato.	PPO genes-POT33	Polyphenol oxidase (PPO) is the major cause of enzymic browning in potato. The browning reaction only occurs as a result of tuber damage leading to a loss of this subcellular compartmentation. PPO catalyzes the conversion of monophenols polyphenols to o-dihydroxyphenols or to quinones. The quinone products can then polymerize and react with amino acid groups of cellular proteins, resulting in brown or black pigment deposits. Such damage causes considerable economic and nutritional losses in the process of potato.	Coetzer *et al*., 2001; Song, 2009
Brassica	Transgenic with 90% reduction of sinapine and a higher level of choline in seeds	Ferulic acid 5-hydroxylase (FAH) and sinapoylglu-cose: choline sinapoyltra n-sferase (SCT).	Sinapine (sinapoylcholine) is the most abundant anti-nutritional phenolic compound in seeds of cruciferous species and therefore is a target for elimination in canola (*Brassica napus*) seeds. Sinapine causes an unpleasant flavor in the meat and milk of animals fed on canola/B. napus (Pearson *et al*., 1980), and excessive consumption of sinapine may cause serious growth and reproductive problems.	Bhinu *et al*., 2009
	Higher levels of lutein, -carotene and zeaxanthein	DE-ETIOLATED1	DET1 suppression in *B. napus* can increase the levels of carotenoids and reduce the levels of sinapate	Wei *et al*., 2009

		(DET1)	esters simultaneously in the seeds, thus enhancing their overall nutritional value.	
	Increased oleic acid composition in transgenic plants of *Brassica napus.*	Brassica napus FAD2 gene (BnFAD2)	Oleate-Δ12 desaturase (FAD2) is a key enzyme involved in the conversion of oleic acid (C18:1) into linoleic acid (C18:2). BnFAD2 gene was efficiently down-regulated and mediated by its RNAi gene, and oleic acid composition in transgenic rapeseeds was significantly enhanced.	Tian *et al*., 2011
	low glucosinolate	BjMYB28 transcription factor	Improve the seed-meal quality through the development of low-seed-glucosinolate lines (<30 μmol/g DW), as high amounts of certain seed glucosinolates are known to be anti-nutritional and reduce the meal palatability. transgenic B. juncea lines having seed glucosinolates as low as 11.26 μmol/g DW, through RNAi-based targeted suppression of BjMYB28,this results indicate that targeted silencing of a key glucosinolate transcriptional regulator MYB28 has huge potential for reducing the glucosinolates content and improving the seed-meal quality of oilseed Brassica crops.	Augustine *et al*., 2013
Cotton	High oleic and stearic acid content in cottonseed oil	Stearoyl-acyl-carrier protein Delta 9-desaturase (ghSAD-1) and oleoyl-phosphatidylcholine omega 6-desaturase (ghFAD2-1)	The silencing of ghSAD-1 and/or ghFAD2-1 to various degrees enables the development of cottonseed oils having novel combinations of palmitic, stearic, oleic, and linoleic contents that can be used in margarines and deep frying without hydrogenation and also potentially in high-value confectionery applications.	Liu *et al*., 2002
	Production of ultra-low gossypol cottonseed (ULGCS)	δ-cadinene synthase	RNAi-knockdown of -cadinene synthase genes was used to engineer plants that produced ultra-low gossypol cottonseed (ULGCS).	Rathore *et al*., 2011

	Reduction of toxic gossypol in cotton seed	delta-cadinene synthase	By using RNAi to disrupt gossypol biosynthesis in cottonseed tissue by interfering with the expression of the δ-cadinene synthase gene during seed development. Targeted genetic modification, applied to an underutilized agricultural byproduct, provides a mechanism to open up a new source of nutrition for hundreds of millions of people.	Sunilkumar *et al*., 2006
Sorghum	Modulation of kernel storage proteins in grain sorghum.	γ- and the 29-kDa α-kafirins	Down-regulation of c-kafirin alone does not alter protein body formation or impacts protein digestibility of cooked flour samples. However, reduction in accumulation of a predicted 29-kDa a-kafirin alters the morphology of protein body and enhances protein digestibility in both raw and cooked samples.	Kumar *et al*., 2012
	Lysin improvement in sorghum	Three lysine-deficient kafirins(ä-kaf-2, ã-kaf-1 and -2)	The transgenic co-suppression of the target kafirins resulted in Suppression of three lysine-poor storage proteins which observed improve lysine and also change endosperm structural from a hard, corneous endosperm to a soft, floury endosperm.	Grootboom, 2010
Soybean	Low alpha-linolenic acids (18:3) of fad3-mutant phenotype in soybean [*Glycine max* (Merr.)]	GmFAD3	The FAD3 enzyme is responsible for the synthesis of alpha-linolenic acids (18:3) in the polyunsaturated fatty acid pathway. It is this fatty acid that contributes mostly to the instability of soybean and other seed oils. Therefore, a significant reduction of this fatty acid will increase the stability of the seed oil, enhancing the seed agronomical value.	Flores *et al*., 2008
Carrot	Reduced allergenicity to patients with carrot allergy.	Dau c 1.01 and Dau c 1.02	The decrease of the allergenic potential in Dau c 1-silenced plants was sufficient to cause a reduced allergenic reactivity in patients with carrot allergy, as determined with skin prick tests (SPT).	Peters *et al*., 2011
Apple	Fruits with decreased allergenicity	Mal d 1	Apple allergy is dominated by IgE antibodies against Mal d 1 in areas where birch pollen is endemic.	Gilissen *et al*., 2005

			Apples with significantly decreased levels of Mal d 1 would allow most patients in these areas to eat apples without allergic reactions.	
		Mal d 1	These levels of silencing were unaffected by grafting, and have been stable over more than 3 years, and throughout all developmental stages.	Krath *et al*., 2009
Coffee	Decaffeinated coffee plants	Theobromine synthase (CaMXMT 1)	The demand for decaffeinated coffee is increasing because the stimulatory effects of caffeine can adversely affect sensitive individuals by triggering palpitations, increased blood pressure and insomnia.	Ogita *et al*., 2003
Barley	Increased amylose content in endosperm of barley seeds	Starch branching enzyme (SBE IIa or SBE IIb)	High-amylose wheat has a significant potential to improve human health (Protection from several disease like Colon cancer, diabetes, obesity, osteoporosis & Cardiovascular Disease) through its resistant starch content	Regina *et al*., 2010

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