Annals of Agricultural & Crop Sciences

Open Access

Austin Publishing Group

Special Article - Agricultural Biotechnology

Application of Agricultural Biotechnology for High Nutritious Food Products

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Received: November 12, 2018; **Accepted:** December 12, 2018; **Published:** December 19, 2018

Abstract

Agricultural biotechnology has some controversy impacts on global economy and international regulations. But, it has enhanced the production of crops and foods with high nutritious. Some time, it has not secured human and environmental safety, intellectual property rights, consumer choice, ethics, food security, poverty reduction and environmental conservation. Even though, it has provided benefits to agricultural producers with improved safety and wholesomeness. Engineered plants have helped in improved crop yields per acre with insect or pests resistance cultivars *via* less of utilization of herbicides, pesticides, and water and tilling. Reduced pest damage in transgenic corn can express the genes to control insect or pests at lower levels of mycotoxins (a carcinogen). Biotechnological methodologies and development has helped in genetically modification of crops with proper organizations, rules and regulations to ensure food safety. Nutritionally improved golden rice, biotech brinjal, late blight-resistant potato or potato ring and spot virus-resistant papaya are Genetically Modified (GM) crops for commercial cultivation. Improvement of agronomic traits (yield and abiotic stress resistance) has shown in improved biomass feedstock's, nutrition and food functionality as well as production factories for therapeutics and industrial products. Biofortification is feasible and cost-effective techniques for delivering micronutrient with improved nutrition globally. Increasing iron, zinc and β-carotene (provitamin A) content in staple foods can be achieved by conventional plant breeding and genetic engineering tools as provided the improved micronutrient composition in plant foods. In this proposed paper author will discuss about techniques to improve nutrient in foods.

Keywords: Agricultural biotechnology; Micronutrient; Improved safety; Biofortification; Plant breeding

Abbreviations

BT: *Bacillus Thuringiensis*; CRISPR/CAS: Clustered Regularly Interspaced Short Palindromic Repeat/CRISPR-Associated Protein; DMA: Deoxymugeneic Acid; DNA: Deoxyribonucleic Acid; Fe: Iron/ Ferric; Flp: Recombinase Flippase; GM: Genetically Modified; IRT: Iron Regulated Transporter; Lys: Lysine; Met: Methionine; Mn: Manganese; NA: Nicotinamide; QTLs: Quantitative Trait Loci; SSN: Site-Specific Nuclease; SSR: Site-Specific Recombinase; TALENs: Transcriptional Activator-Like Effector Nucleases; Trp: Tryptophan; ZFNs: Zinc Finger Nucleases; Zn: Zinc

Introduction

Crop plants can provide essential food nutrients (carbohydrates, lipids, proteins, minerals and vitamins), directly or indirectly to humans and livestock. The level and composition of food nutrients is found to vary in different food crops. Some plant foods are often found to deficient in certain nutrient components. So single food crop cannot provide balanced diet to people and malnutrition and deficiency diseases are frequently reported in developing countries due to poverty conditions. So, development and application of biotechnology processes can provide novel opportunities and possibilities to enhance the nutritional quality in crops *via* creating necessary genetic modification. Aagricultural biotechnology has provides herbicide tolerance, insect resistance and virus resistance crop food with increasing effort and promising proof-of-concept products. As result, output traits have enhanced the nutritional quality of crops. Advancements in plant transformation and transgene expression in plants have produced a variety of bio-products at large scale and low cost [1]. Synthetic asp1 has been expressed normally in the transgenic tobacco leaves and is reported the high level ASP1 protein accumulation with increased levels of total amino acids and protein in transgenic plants. The *asp1* gene has been also transformed and expressed in the leaves and primary roots of cassava plants with elevated content of some amino acids with no significant difference in the protein content of leaves [2]. Genome editing is comprised of wide variety of tools using a Site-Specific Recombinase (SSR) or a Site-Specific Nuclease (SSN) system *via* helping in recognition of a known sequence. SSN system has generated single or double strand DNA breaks and activates endogenous DNA repair pathways. SSR technology (Cre/loxP and Flp/FRT) are able to knockdown or knock-in genes in the genome of eukaryotes, which is dependent on the orientation of the specific sites (loxP or FLP) flanking the target site. SSN is developed to cleave genomic sequences, mega-nucleases (homing endonuclease), Zinc Finger Nucleases (ZFNs), Transcriptional Activator-Like Effector Nucleases (TALENs), and the CRISPR/Cas nuclease system (Clustered Regularly Interspaced Short

Citation: Srivastava RK. Application of Agricultural Biotechnology for High Nutritious Food Products. Ann Agric Crop Sci. 2018; 3(2): 1038.

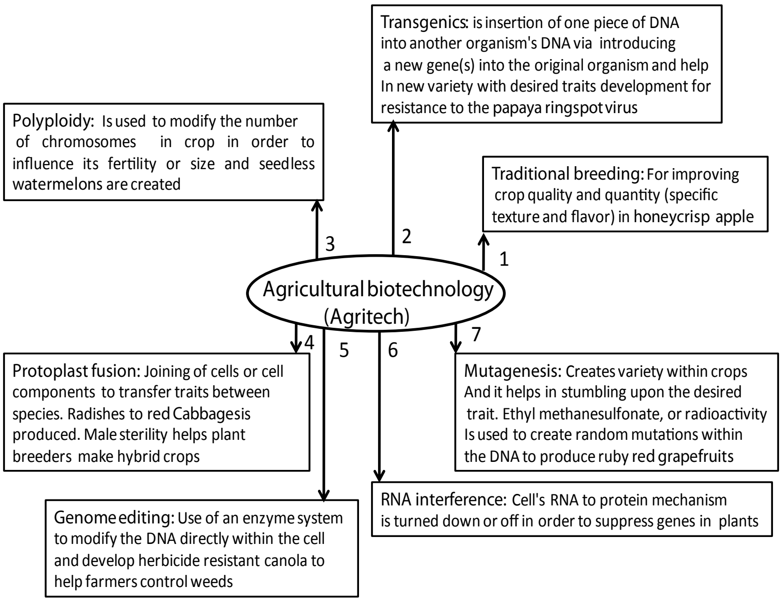

Figure 1: Different strategies of agricultural biotechnology for improving the nutritional quantity and disease resistance in plant food products [15,16].

Palindromic Repeat/CRISPR-associated protein) [3]. Plant breeding has been helped us to enhance the improved varieties of plants and their products development (seeds or fruits or any others parts having food storage capability) with more nutritious contents and shown its *in vitro* development with better adaption to a specific environment. It has improved possibilities for the storage and transportation of germplasm with easy the availability of seed material throughout the year in all the seasons [4]. This technique has helped in rapid adaptation and selection of cultivars with more resistant to specific stress factors (for instance, salinity and acid soils) and also helped in production of healthy cultivars (due to rapid reproduction and multiplication) which is free of viruses and pathogenic agents. Increased food production and improving food processing is only possible by applying biotechnological innovative techniques. Plant biotechnology has helped in improving food quantity with nutrition (such as vitamin A, iron, zinc, protein and essential amino acids, and essential fatty acids) and reduced levels of ant nutritional factors (such as cyanogen's, phytates, and glycoalkaloid); and increased levels of factors in developing countries. It has influenced bioavailability and utilization of essential nutrients (such as cysteine residues) *via* advancing through field trial stage and regulatory processes towards commercialization [5]. Development and application of modern agricultural biotechnology has helped in development and production of genetically modified crops for food and fodder crops. It has helped in gene discovery, genetic transformation and development of genetically modified crops systems to comply with the world regulatory framework on bio-safety as big challenges which are faced by many countries in agriculture sector. It can help in first generation biotech crops with their released for production [6]. Biotechnology strategies have been used to improve the amount and availability of nutrients in plant crops and main strategies are simple plant selection for varieties with high nutrient density in the seeds. Cross-breeding can incorporate a desired trait within a plant whereas genetic engineering is utilized to manipulate the nutrient content of the plant. In plant, cross-breeding technique has helped in combining of all genes of the parent plants and the parent progeny have both desirable and undesirable traits. It has helped in elimination of undesirable traits *via* plant breeders (back-cross) for new plant varieties in over several generations. Hybridization has been used to create varieties of low-phytate corn, barley, and rice. Genetic engineering has helped in the gene(s) encoding for a desired trait(s) in a plant *via* introduction in a precise and controlled manner within a relatively short period of time. And golden rice is good example and is rich in carotenoids with higher amounts of iron. Genetically engineered plants have improved nutrition with tremendous potential for revolutionizing nutrition [7]. Author will discuss important of agriculture biotechnology and these has helped in enhancement of nutrient in plant products.

Agriculture Biotechnology

It includes the use of biological processes or organisms for the improvement of the characteristics of higher plants. It helps in modification and enhancement of living organisms at the molecular level, frequently under modern biotechnology. It is more focus on recent developments on techniques or methods used in the seed industry [8]. It has shown their contribution in following ways:

- It helps in speed-up the multiplication process for vegetatively propagated crops and ensures the detection of diseases transmitted by seed or planting material.
- It helps in testing of varietal identity and purity with protection of seed with biological control agent. It eradicates diseases transmitted by planting material.

Modern tools and applications of biotechnology has also helped in improvement of diatoms plant cultivation and diatoms have shown decisive role in the ecosystem from millions of years as oxygen synthesizers on earth and also most important sources of biomass in oceans. Diatoms plant is decisively useful in commercial and industrial applications as the carbon neutral synthesis of fuels, pharmaceuticals,

Table 1: Various strategies utilized in various plant products with enhanced nutritious components.

Biotech Strategy	Food products	Enhanced nutrient	Reference
1- Metabolic engineering	Biofortified rice	Folate (vitamin B) stability	[17]
2- Plant biotechnology (*via* conventional **plant breeding and genetic engineering** as sustainable strategy)	Staple foods	Improving iron, zinc and vitamin A	[18]
3- Introduction of multiple iron homeostasis genes for expression of the iron storage protein ferritin using **endosperm-specific promoters**	Biofortified rice	Iron nutrition (sixfold higher) for iron-deficiency anemia in human population. Accumulation of zinc up to 1.6-times in transgenic seed in the field.	[19]
4- Application of Iron Regulated Transporter (IRT) in addition to Fe(III)-phytosiderophore chelates with help of Arabidopsis (AtI) RT1 under the control of MsENOD12B promoter *via* **genetic engineering approaches**	Rice a staple food	Increased iron content	[20]
5- Editing cis acting regulatory elements in promoter region has shifted the expression of transporters and thiamine binding proteins to endosperm through Pathway Editing Targets gene *via* **metabolic engineering**	Biofortification in Rice Grains	Thiamine binding proteins to endosperm is reported and effect of overexpression of a few genes such as *thi*1 and *thi*C, has helped in thiamine accumulation in rice	[21]
6- Proteins can catalyse chloride-efflux out of the roots or that restrict xylem loading *via* genetic engineering for more resistant crop. **Nitrate fertilization strategy** can suppress uptake of chloride by means of an antagonistic anion-anion uptake competition.	In wheat for high yield and quality formation	High concentrations of chloride in the soil can help to increase phyto-availability of the heavy metal cadmium, accumulating in wheat. Chloride ion can effect quality of plant-based products by conferring a salty taste that decreases market appeal of e.g. fruit juices and beverages.	[22]
7- Expression in Arabidopsis Nicotianamine synthase 1 (*AtNAS*1), bean Ferritin (Pvferritin), bacterial Carotene desaturase (*CRTI*) and maize Phytoene synthase (*ZmPSY*) in **single genetic locus** is sustainable and effective approach	In rice endosperm grains	Improvement of iron, zinc and β-carotene content	[23]
8-Nicotianamine synthase (utilizes *OsNAS*2 or Pv *Ferrtin*, or both genes) expression as single genes as well as in combination is used. Ferritin is important for iron storage in plants because it can store up to 4500 iron ions.	In wheat	Improves dietary iron and zinc levels. NAS catalyzes the biosynthesis of nicotianamine (NA), which is a precursor of the iron chelator deoxymugeneic acid (DMA) required for long distance iron translocation	[24]
9-Rice breeding program helps in genes and quantitative trait loci (QTLs) as design a desirable genotype with superior in multiple grain quality traits involved in enhancement of micronutrient element and amino acids	Rice grain nutritional traits	Grain protein and amino acid content, vitamins and minerals, glycemic index value, phenolic and flavonoid compounds, phytic acid, zinc and iron content	[25]
10- ZIP transporter gene (OsZIP3) is used for protein localization, gene expression patterns, transport mechanism, metal ion interaction, and natural allelic variations	In rice	Zinc and iron as micronutrients for human health. Zinc-regulated transporters and iron-regulated transporter-like proteins control the absorption and translocation of Zn and Fe and maintain their homeostasis in rice	[26]

health foods, biomolecules, materials relevant to nanotechnology and bioremediations of contaminated water. Agriculture biotechnology has helped in progress in the technologies of diatom molecular biology as model organisms and culturing conditions at photo bioreactor level for more efficiency [8]. With help of agricultural biotechnology, crop plants have succeeded to provide more amount of essential food nutrients (carbohydrates, lipids, proteins, minerals and vitamins, directly or indirectly) to humans and livestock and it is shown in (Figure 1). But level and composition of food nutrients can be found more vary in different food crops. Some time, plant foods may be deficient in certain nutrient components which cannot provide the balanced diet to human and causes malnutrition and deficiency diseases in the developing countries. Relying on a single food crop as source of nutrients will causes more malnutrition and deficiency diseases and it is due to mainly to poverty in the developing countries. The development and application of biotechnology has offered the opportunities and novel possibilities to enhance the nutritional quality of crops [9]. Necessary genetic variability is the emphasis from agricultural biotechnology and has placed the desired or necessary input traits of crops (herbicide tolerance, insect resistance and virus resistance). Agricultural biotechnology has put more effort for promising proof-of-concept products and shown the desired output traits (enhancing the nutritional quality) of crops from last many decades. Advancements in plant transformation and transgene expression have been used in plants as bioreactors to produce a variety of bio-products at large scale and low cost which has helped to provide the plant-derived healthcare products atl commercialized level [10,11].

Continuously increasing demand for agricultural production has been reported with urgent need for improved the nutritional quality of human diets in rapidly growing human population. Malnutrition is reported as critical health issue at worldwide and macronutrient and micronutrient deficiencies diet of human population are prevalent reason in developing countries. And over-consumption of certain nutrients in developed countries (e.g., saturated fatty acids) has shown high incidence of certain chronic diseases. Declining natural resources such as arable land and water are big challenges to humans for production of huge quantity of agriculture food product with rich of nutrient without degrading the environment. Biotechnology has offered a valuable tool to help achieve these goals and advances in biotechnology can have more promise to improve human nutrition by enhancing the nutrient density of plant foods. Issues relating to the safety of food products from genetically engineered crops are also need to solve [12]. Plant-based foods are the array of nutrients and are essential for human nutrition and promote good health. Major staple crops of the world are found to deficient in some of essential nutrients. Traditional agricultural approaches can be marginally enhanced the nutritional value to some plant foods. But advances in molecular biology are applied rapidly for engineering crops for enhanced key nutrients. Nutritional targets are elevated mineral content, improved fatty acid composition, increased amino acid levels and heightened antioxidant levels. It needs to fortify the crops for getting the benefits to human nutrition [13]. Plants have helped to provide most of the nutrients required in the human in major staple crops and shown in (Table 1). Some staple crops are found to deficient in some of essential nutrients (micronutrients like vitamin A, iron and zinc) and it has caused malnutrition more than 40% of the world's population. Advances in molecular biology are exploited to produce crops with enhancement in micronutrients (like vitamin A, iron and zinc key nutrients). Other nutritional targets are also got in modification of fatty acid composition and the enhancement of antioxidant levels (carotenoids, such as lycopene, and flavonoids). These bio fortified crops are required for improved human nutrition [14]. Author will discuss some of recent development on food products with help of agricultural biotechnology which is reported to have enhanced nutrition and shown below.

Modified proteins in crops

Plant breeders have improved the quality and yield for major crops with maintenance of the safety of the food supply. Biochemical mechanisms are used to determine the selected traits. In a crossing over processes, tens of thousands of genes are mixed and reasserted, largely at random. And it helps in selecting the lines to be crossed and recognizing the preferred progeny. With of recombinant DNA technology, breeders can be extended the range of biological materials with their genes assessment. It has gained new insights into genome organization and gene structure as well as the nature and function of the proteins that those genes encode. Specificity in altering the genetic makeup of new crop varieties can be possible in easy way. Resistance to insect pests can be achieved by addition of a single well-characterized gene [27]. Coding for uncharacterized and possible toxic proteins can be eliminated by generations of backcrossing and screening to recover a commercially acceptable insect-resistant line. Unique opportunities to identify the individual components of foods is possible that help in identification of allergies and it can be removed from food, or change them, so that the food can be consumed safely. Genetic engineering has helped to provide a number of commercial products and it should be approved through regulatory processes to address the environmental and food safety concerns. Major shortages of basic nutrients to a diet is reported exclusively on staple foods include vitamin A, iron, iodine, zinc, and quality protein, and deficiencies in these nutrients can cause micronutrient and protein-energy malnutrition *via* consuming such foods in the developing world. Enhancing the nutritional quality of crops can be helped in future food security and the nutritional well-being of world population [27,28]. Humans and animals cannot synthesize essential amino acids (branch chain amino acids, Methionine (Met), Lysine (Lys) and Tryptophan (Trp)). And these amino acids are needed to be supplied through the proper diets. In non-balanced diet or food, several essential amino acids are reported to deficient or completely lacking which are taken such crops with above mentioned essential component and used for human food and animal feed. Soybean is reported to deficient in Met and maize crops are lacking in Lys and Trp amino acids. Roles of essential amino acids in animal nutrition are important in their diet and amino acids deficient in various plants and their biosynthesis pathways are needed to improve the availability of essential amino acids in plants through genetic engineering approach [29]. Humans and farm animals has shown their incapability for synthesis of number of essential amino acids, critical for their survival. And for them it is required these essential amino acids *via* their diets. Cereal and legume crops are major food and feed sources for humans and livestock at worldwide and they possess limiting levels of some of these essential amino acids (particularly Lys and Met) [12]. Extensive efforts can be helped in fortifying crop plants with these essential amino acids using traditional breeding and mutagenesis. And maize is shown in successes from mentioned strategies. Additional efforts using genetic engineering approaches can be helped in increasing the synthesis as well as reducing the catabolism of these essential amino acids *via* respective expression of genes for recombinant proteins enriched in them. Basic biological aspects associated with the synthesis and accumulation of these amino acids in plants can be described the recent developments associated with the fortification of crop plants with essential amino acids by genetic engineering approaches [30]. Horticultural crops (particularly fruits and vegetables) have been reported to provide the numerous health compounds (vitamins, antioxidants, and amino acids). Most essential amino acids are reported to not produce by the organism and needed to obtain from their diet (meat, eggs, and milk, as well as a variety of plants). Genetic resources for plant breeding technique can help in providing high essential amino acid content which is generally limited its plant growth. Biosynthetic pathways of essential amino acids and their interactions can be understood by regulatory networks in plants *via* use genetic engineering which can improve the essential amino acid content of horticultural plants. It can render these plants with more nutritionally favorable crops and also possible future directions towards their bio-fortification [31,32].

Genetically modified crops/foods

Genetic modification is done by set of gene technology *via* altering the genetic machinery in living organisms (plants or microorganisms). Combining genes of different plants strain is achieved easily *via* recombinant DNA technology and Genetically Modified (GM) crops or foods are synthesized. Transgenic crops are grown commercially in field and are basically herbicide and insecticide resistant crops and soybeans, corn, cotton and canola are the best examples. Sweet potato are found to resistant to a virus (destroy most of the African harvest). Rice with increased iron and vitamins are also product of Genetically Modified (GM) crops and it has alleviated the chronic malnutrition in Asian countries. And a variety of genetically modified plants have capability to survive weather extremes [33]. Genetically Modified (GM) bananas has produced human vaccines against infectious diseases (hepatitis B) and fish has found to mature more quickly. Many fruit and nut trees has produced more yield per years compared to earlier. Some plants can produce new plastics with unique properties. Controversies and public concern for GM foods and crops are reported on human and environmental safety, labeling and consumer choice, intellectual property rights, ethics, food security, poverty reduction and environmental conservation which is needed to solve [34].

Fortified rice (a staple crop)

Biofortification process has helped us for increasing the contents of vitamins and minerals in our food crops *via* plant breeding or biotechnology technique. Micronutrient elements fortification have shown more potential to combat widespread micronutrient deficiencies in rice food (*Oryza sativa* L.) as utilized as humans feeds and more than half of the world's population has been used it as a staple food in many parts of Asia. Micronutrient transport in rice is controlled at several stages (uptake from soil, transport from root to shoot, careful control of subcellular micronutrient transport, and most importantly transport to seeds) [35]. To enhance micronutrient accumulation (Fe, Zn, and Mn) in rice seeds, carefully regulation all of these process is required. Increasing the contents/expression of genes is required to encode metal chelators (mostly phytosiderophores) and metal transporters, Fe storage protein ferritin and phytase and these can help in significantly increased the micronutrient content of rice. Plant transformation has offered great opportunities for increasing the amounts of trace metals in the endosperm of rice [36]. Improving in nutritional characteristics of rice can done by using biotechnology *via* helping in production of β-carotene in the rice endosperm. It is possible by introduction of a heat-stable phytase gene and it has increased iron concentration. Plant breeding is conventional techniques and shown the potential usefulness in providing more nutritious in rice like food staples. Plant-breeding is more cost-effective and capability to measure the multiplier effects over time and space, as compared to other supplementation and fortification techniques. Plant breeding in biotechnology is found superior as conventional techniques. In rice, adding β-carotene-related and heat-stable phytase genes are reported. For increasing mineral concentration, it can be applied more quickly. It has no serious negative agronomic consequences and consumers can accept any changes in the colour, taste, texture, and cooking qualities. It has resulted in a measurable improvement in the nutritional status of the malnourished target population [37].

Transgenic vegetables and fruits

Vegetables and fruits has important role in human diets due to rich source of vitamins, minerals, dietary fiber, and phytochemicals. It is associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Vegetable and fruit production is stressed by many biotic stresses (pathogens, pests, and weeds) and required in high amounts of plant protection products per hectare. United States vegetables farmers are growing transgenic squash cultivars resistant to Zucchini yellow mosaic virus whereas watermelon mosaic virus and Cucumber mosaic virus, are regulated and commercialized from many decades [38]. Bt-sweet corn is proven more effective to control of some lepidopteran species *via* accepting in the fresh market in the USA. Bt-fresh-market sweet corn hybrids are released almost every year. Transgenic Bt-eggplant bred are found to reduce pesticide use by farmers in Bangladesh. Transgenic papaya cultivars has carried the coat-protein gene provide effective protection against Papaya ring spot virus. Transgenic plant breeding has provided genetically enhanced seed embedded technology and it has contributed in integration of pest management in horticulture *via* reduction in pesticide sprays with improved food safety *via* minimized pesticide residues. Herbicide-tolerant transgenic crops can reduce plough in fields, *via* saving fuel for less tractor use and also more protection of the structure of the soil *via* reduced erosion. Transgenic vegetable and fruit crops can be important contributions to sustainable vegetable production with more nutritious and healthy food supply [38,39].

Conclusion

Agricultural biotechnology has shown important role in enhancement of high nutrition contents in many crops products and it is available as foods for worldwide. Improved crop yields per acre have reported by insect or pests resistance transgenic plants with least utilization of herbicides, pesticides, water and tilling. Nutritionally improved golden rice, biotech brinjal, late blight-resistant potato or potato ring and spot virus-resistant papaya are reported as best examples of Genetically Modified (GM) crops for commercial cultivation purposes. Improvement of yield and abiotic stress resistance are shown in improved biomass feedstocks, nutrition food as well as food functionality or production factories for therapeutics and industrial products.

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Ann Agric Crop Sci - Volume 3 Issue 2 - 2018
ISSN: 2573-3583 | www.austinpublishinggroup.com

Citation: Srivastava RK. Application of Agricultural Biotechnology for High Nutritious Food Products. Ann Agric Crop Sci. 2018; 3(2): 1038.