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Advances in enhancement of nutraceutical values through Omics technologies in vegetables

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Abstract
The human diet heavily relies on vegetables as a significant source of biologically active nutraceuticals. It has lately been investigated as a viable alternative for the management and prevention of numerous ailments. Due to their safety, effectiveness, possible nutritional value, and therapeutic effects, they have attracted a lot of interest in the diet. Phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids, and anthocyanidins are a few examples of nutraceuticals. They have a significant influence on the healthcare system and may offer medical advantages, such as the diagnosis and treatment of diseases and physiological abnormalities. In order to breed cultivars with better nutritional qualities, it is necessary to identify the plant compounds that are significant for human nutrition. This can be done using both traditional and molecular breeding techniques. The attempts made using traditional breeding techniques have been ineffective and slow. Nutraceutical breeding is becoming simpler as a result of the recent advancements in high throughput omics technologies. Omics technologies indicate a comprehensive method for characterizing and quantifying a biological organism's genes, transcripts, proteins, and metabolites, which are referred to as genomics, transcriptomics, proteomics, and metabolomics, respectively. In order to enhance the nutraceutical qualities of vegetables, high throughput omics techniques along with bioinformatics tools are therefore needed. These techniques also hold tremendous potential for lowering the time and expense associated with quality breeding.

Keywords: Genomics, metabolomics, nutraceuticals, omics, transcriptomics

1. Introduction
The nutritional micronutrients (minerals, vitamins), antioxidants, and useful phytochemicals, or "nutraceuticals," found in vegetable crops are abundant. Some of the elements, such as -carotene, iron, calcium, and folic acid, are of public health concern, which captivates the interest of breeders to boost their content in food sources, particularly vegetable crops, in order to raise dietary intake and decrease deficiency issues. They contribute biologically active secondary metabolites that protect against a variety of diseases, especially non-communicable ones. Vitamins, carotenoids, phenolics, alkaloids, flavonoids, compounds containing nitrogen, organo-sulfur compounds, etc. are among the substances in vegetables that provide health benefits. A number of nutraceutical ingredients, including anthocyanin, beta-carotene, and lycopene, have been found to have positive effects on health. By lowering production and/or compressing the reactive free radicals, these secondary metabolites function as antioxidants in the human body and contribute to homeostatic equilibrium. These substances are regarded as natural nutraceuticals because of their functional significance in maintaining human health. As a result, vegetables high in antioxidants have significant promise for treating non-communicable diseases like obesity, cardio-vascular disease, some types of cancer, phytoestrogens, anti-inflammatory agents, and nutritional inadequacies. Due to the slow and ineffective progress of conventional breeding techniques, advances in sequencing and the accessibility of various omics technologies have created the opportunity to use these tools to improve the nutraceutical qualities of vegetables, thereby ensuring the security of a balanced diet. Crops can be improved using integrated omics methodologies, such as genomes, transcriptomics, proteomics, and metabolomics, along with artificial intelligence and machine learning techniques. This article offers insights into omics research that is done to improve the nutritional value of a wide variety vegetables.

1.1 Nutraceuticals

Dr. Stephen De Felice MD, the founder and chairman of Innovation (FIM) in Medicine, combined the terms "nutrition" and "pharmaceutical" to create the term "nutraceutical" in 1989. Nutraceutical is equal to nutrition plus pharmaceutical. Any substance that is a food or a component of food that has therapeutic or health advantages, including the prevention and treatment of disease, is a nutraceutical, in Dr. De Felice's words. "Any non-toxic dietary component with scientifically demonstrated health benefits, including illness treatment or prevention" is the definition of a nutraceutical (Nayak *et al*., 2021) [16].

1.2 Nutraceuticals in vegetables

A healthy diet must include vegetables since they supply a large portion of the nutritional need for vitamins and minerals. One of the key components of quality characteristics in vegetable crops is nutraceuticals. Bioactives and nutraceuticals are important in the prevention of cancer, heart disease, and stroke. Lycopene, found in tomatoes, watermelons, carrots, and red peppers, prevents prostate cancer. Ascorbic acid, found in green peppers, broccoli, green leafy vegetables, cabbage, and tomatoes, aids in the growth, development, and repair of all body tissues as well as the absorption of iron from food sources. Momordicin and charantin, two compounds found in bitter gourd, have anti-diabetic and blood-purifying activities. The majority of vegetables are naturally low in fat and calories, and none of them contain cholesterol, making them good for heart. Dietary fibre improves intestinal function, lowers risk of heart disease, and lowers blood cholesterol levels. India is blessed with an abundance of cultivated and wild vegetable crops, many of which are rich in components thought to provide a variety of health benefits. The immune system is strengthened by allyl sulphides found in Allium vegetables (garlic, onions, chives, and leeks), and glucosinolates are antioxidants and nutritional minerals found in Cole crops. Vegetable species vary greatly, with tomato having antioxidants and lycopene, chilli having capsaicin, watermelon having lycopene, and broccoli and cauliflower having glucosinolates. Environmental factors and quantitative trait loci (QTLs) primarily regulate the metabolic pathway for the synthesis of the antioxidant molecules. Vegetable crop germplasm has very little information on such QTLs, and addressing such complex features requires the use of increasingly sophisticated molecular and genomics tools and methodologies (Singh *et al*., 2021) [29]. Environmental factors and quantitative trait loci (QTLs) primarily regulate the metabolic pathway for the synthesis of the antioxidant molecules. Vegetable crop germplasm has very little information on such QTLs, and addressing such complex features requires the use of increasingly sophisticated molecular and genomics tools and methodologies (Singh *et al*., 2020) [28].

1.3 Need for improving the nutraceuticals

It is crucial to produce varieties with high yield, superior quality qualities, and nutraceutical properties because the emphasis on breeding for yield traits negatively impacted quality attributes. Therefore, improving the nutraceutical qualities of vegetable crops requires specific consideration. Nutraceuticals offer excellent advantages for health. Due to their possible health benefits, consumers pay close attention to carotenoids, anthocyanins, flavonoids, glucosinolates, capsaicin, oleoresins, and terpenoids (Behara *et al*., 2019).

1.4 The central dogma of molecular biology

The central dogma depicts the replication of DNA, the transcription of DNA into RNA, and the translation of RNA into proteins as well as the flow of genetic information inside cells. Through the framework, the idea of a series of interactions can be comprehended. Among the most prevalent are biopolymers. Proteins, RNA, and DNA make up the main group of biopolymers; these three are further broken down into general transfers, unidentified transfers, and special transfers. In a rare circumstance, special transfers take place in the lab. Nearly every cell transfers information generally. It speaks of the consistent exchange of information via transcription and translation. It is stated that unknown transfers never happen.

2. Omics

The term "omics" refers to the study of massive collections of biological molecules in a biological environment. The understanding that complex biological processes are not solely regulated by DNA spurred the rapid growth of various specialties in molecular biology, together referred to as OMICS. In OMICS, there are many measures taken for each endpoint. Data integration from OMICS: The identification of the true causes and states of disease, which is primarily done by software, can frequently be facilitated by the effective integration of data from various OMICS. Several biological disciplines with the suffix -omics in their names, such as genomics, proteomics, metabolomics, metagenomics, phenomics, and transcriptomics, are the branches of science popularly referred to as omics. Omics aims at the collective characterisation and quantification of pools of biological molecules that translate into the structure, function and dynamics of an organism or organisms (Tahir ul Qamar *et al*., 2020) [31].

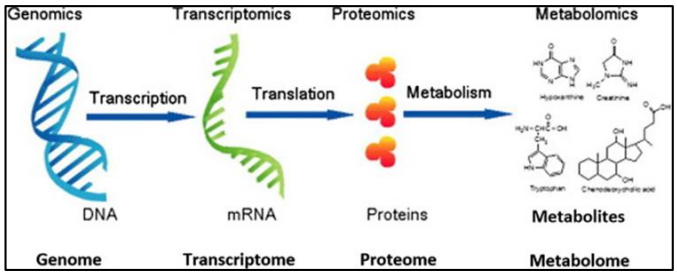

Fig 1: An overview of omics approaches (Figure is taken from Frueh and Burczynski, 2021).

2.1 Genomics

The genome is totality of a cell's genetic material. The process of deciphering the four nucleotides—adenine (A), guanine (G), cytosine (C), and thymine (T)-that make up the genetic code in a DNA sample is known as genome sequencing. Although there are other techniques for sequencing DNA, the dideoxy or chain termination method, introduced by Sanger *et al*. in 1977 [22], and the shotgun sequencing technique are the most well-known and often employed techniques. First generation sequencing, second generation sequencing, and third generation sequencing are the three main stages of evolution of genome sequencing (Heather and Chain, 2016) [10]. Various sequencing methods, such as first, second and third generation sequencing, were used at various stages of

genome sequencing.

Dideoxy or Chain Termination Method: Also referred to as the Sanger method, this technique has been automated and has undergone a number of improvements since it was first developed. Sequencers are currently utilized for large-scale DNA sequencing (Setia *et al*., 2008) [25]. In order to sequence vast amounts of DNA more quickly, automated sequencing has been developed. However, the new methods are founded on the same ideas as Sanger's approach (Tahir ul Qamar *et al*., 2020) [31].

Shotgun sequencing method: It is a frequently used technique for quickly determining an organism's whole genome and is especially well adapted to high-throughput assembly-line style methodologies.

Polymerase Chain Reaction: Mullis *et al*. (1986) [15] achieved a huge advancement in the field of molecular biology by developing the polymerase chain reaction (PCR). This method allows for the rapid amplification of target DNA sequences that are initially present in infinitesimally small quantities in a population of other DNA molecules. It also allows for the generation of numerous copies of a particular DNA sequence through a series of in vitro reactions.

Genome Annotation: Once the genome sequence is available, it should be annotated, which means finding the potential genes and assigning functions to them. Most of this is done in silico, i.e., with the aid of computer programs. Function assignment relies extensively on sequence similarity. This means that a function is assigned to a gene in a newly sequenced genome based on its similarity. to the already available gene sequences in sequence databases, such as GenBank. The most widely used similarity detection tool is the BLAST programme (Setubal and da Silva, 2004). The classification of genes is done according to their assigned function. Generally, each genome project develops its own classification scheme, but most are based on the one originally developed for *Escherichia coli* bacterium.

2.1.1 Applications of genomics

The genome-wide association analyses give a more comprehensive picture of how all genes function and interact. The development of genomic technologies has made it possible for us to create horticultural crops with highly advantageous economic features. Crop biotechnology has used genome sequencing, subsequent functional annotation, and molecular analysis in a variety of applications (Shalini *et al*., 2018) [27]. Gene identification and cloning, gene prediction and discovery, genetic mapping and gene location, genome manipulation, QTL analysis, molecular markers and MAS, comparative genomics, gene banks and chromosome stocks, understanding expression profiles, responses, and interactions are some of the additional applications of genomics.

2.2 Transcriptomics

The information of expressed gene sequences in a particular tissue at a particular moment is provided by the transcriptome. The cell's transcriptome is constantly changing under various situations, and secondly, alternative splicing allows the same gene to produce many transcripts. These two factors make the transcriptome dynamic in nature. Transcript sequencing can be used to investigate the mechanism and control of alternative splicing. Understanding the function of genes, transcription levels, and the molecular processes involved in cellular metabolism are all aided by the study of the transcriptome. The discovery of candidate genes for a specific biological characteristic and the identification of important enzymes in a metabolic pathway are both facilitated by expression analysis of genes. The development of NGS technologies allowed for the affordable acquisition of transcriptome assemblies that are helpful for gene annotation. The analysis of transcriptome assemblies provides details on numerous functional markers, including single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR) associated with stress resistance. By normalizing the data with the aid of statistical modelling after getting the quantitative counts of each transcript, differential gene expression could be examined (Scossa *et al*., 2021) [24].

2.2.1 Applications of Transcriptomics

In order to explain the differences in transcriptome data throughout seed germination, growth, development, and diverse stresses, the microarray technology has been successfully applied. Functional genomics is a combinatorial technique that makes use of expression analysis and genetic data to find potential genes associated with specific biological traits. By using this method, the area of the wheat genome responsible for seed formation has been identified. Crop genomes have been mapped for QTLs associated to grain development and tolerance to biotic and abiotic stressors, and these QTLs have been successfully used to advance crop types. RNA interference (RNAi), mutagenesis, and epigenetics are examples of functional approaches that can be used as gene silencing strategies to improve agricultural crops. A genomic mapping technique called bulk segregate analysis (BSA) is used to locate the chromosomal area associated with the expression of commercially significant agricultural characteristics. This method was successfully used to generate pigeon peas that were resistant to disease. Molecular switches are DNA-binding transcriptional factors with sequence specificity that control biological processes. By comparing newly sequenced plant species' genomes to the TF sequences of model plants, it was possible to identify the transcriptional factors (TF) of those species (*Arabidopsis thaliana*) (Jain *et al*., 2019) [11].

2.3 Proteomics

The identification and description of the entire collection of proteins originating from a genome is known as proteomics. For the protein's structure and key regulatory functions to be sustained, the genetic code is necessary. In proteomics, amino acid sequences are analyzed to determine their relative concentrations and different post-translational modifications. Proteomics is dynamic in nature and sensitive to translation and posttranslational changes, in contrast to genomics. Understanding complex biological processes and cellular reactions to environmental stress is made easier with the help of proteomics research. Since proteomics shows how proteins maintain homeostasis inside cells, participate in cell signaling pathways, and are necessary for structural maintenance, it has become a significant tool for crop improvement (Benkeblia *et al*., 2014) [2]. Gel-based proteomics techniques are the most frequently used methods for high-throughput protein analysis. In gel-based proteomics, there are two primary steps: a separation step (most often 2-DE), and an identification step (MS). A source of energy for ionising the sample, a device to separate the ions depending on their mass/charge ratio, and a

device to detect ions are all components of the mass spectrometer. Shortly after the development of electrospray (ES) and marker-assisted laser desorption ionisation (MALDI), which enable the exact ionisation of big macromolecules, mass spectrometry became a reliable and effective method for proteome characterization. Protein microarrays, also referred to as protein chips, are a sophisticated subset of proteomics that can identify proteins in very little amounts of sample. As they were used in the interaction investigation of the Arabidopsis transcription-factor, these microarrays demonstrated their efficacy as a tool for genome-wide analysis of DNA-protein and protein-protein interaction (Shalini *et al*., 2018) [27].

2.4 Metabolomics

It is the metabolite concentration that gives crops their flavour, aroma, storage ability, and sustainability. Identification and measurement of each molecule in a biological sample are part of the metabolomics process. Based on their existence at the given developmental stage, metabolites are divided into primary and secondary categories. Modern methods including mass spectrometry (MS), nuclear magnetic resonance (NMR), and fourier-transform infrared spectroscopy are improving, making it easier to analyse metabolites (FTIR). The sample extraction at a certain time is necessary for the metabolomics analysis. Our understanding of sophisticated metabolic control and the complexity of biological processes involved in the regulation of plant growth, differentiation, stress tolerance, and pathogen defense mechanisms are made possible by metabolomics approaches. Bioinformatics techniques are used to create large datasets that are then further processed in order to analyse the metabolic network's component parts. The study of metabolomics improves our understanding of intricate molecular networks and complex metabolic pathways. Modern molecular breeding strategies have been developed with the use of computational tools like molecular dynamics simulations and three-dimensional structural modelling (Witzel *et al*., 2015) [35].

2.4.1 Applications of Metabolomics

In order to develop metabolic profiles for the investigation of various stresses, improving economically significant features, and influence of heredity, metabolomics studies have been carried out in the agriculture and horticulture sector. The use of metabolic profiles to assess the effects of seasonal variations, geographic location, and natural variation has been beneficial. Transgenic plants have been examined based on their metabolic profiles. Orthologous enzymes with related catalytic characteristics have been found thanks to metabolic flow studies. Additionally, metabolic investigations have been carried out to characterize the growth profile, developmental stages, and study of chemo taxis. By determining the relative profiles of metabolites, the biochemical network has been built. The combination of metabolome and transcriptome data has prompted research into regulatory networks and the relationship between genetic makeup and phenotypic traits (Sahoo *et al*., 2022) [21].

3. Advanced omics approaches employed for enhancing nutraceuticals

- Molecular breeding
- Genomics by Sequencing (GBS)- SNP's discovery & use in genotyping platforms
- Transgenic approach
- Genome Wide Association Studies (GWAS)
- RNA interference (RNAi)
- DNA directed RNA interference (ddRNAi)
- CRISPR- associated protein-9-nuclease (CRISPR Cas 9)
- TILLING (Target Induced Local Lesions In Genomics) (Behara *et al*., 2019)
- Proteome analysis
- Metabolite profiling

3.1 Genome sequencing and transcriptome analysis

As compared to Sanger technology, NGS technologies have decreased sequencing costs and turnaround times while enabling millions of simultaneous sequencing processes. Plant species are already routinely sequenced using second generation sequencing (massively parallel sequencing) technologies like Roche/454 pyrosequencing and Illumina/Solexa sequencing, and third generation sequencing (also known as long-read sequencing) is actively being developed and incorporated into sequencing projects like PacBio RS (Pacific Biosciences), Helicos (Helicos), or Ion Torrent (Life Technologies). By combining Sanger and next-generation sequencing methods, it is now possible to sequence the majority of crop genomes (with the exception of those with very large and complex genomes) for a relatively low cost. Three loci in the pea, r (ADP-glucose pyrophosphorylase), rb (starch branching enzyme), and bsg (phosphoglucomutase), have mutant alleles that influence the production of starch and sugar (Harrison *et al*. 2000) [8]. Low levels of starch and high levels of sucrose accumulate when the r, rb, or bsg genes are present. Recent research by Liu *et al*. (2015) [13] revealed differential expression of genes associated to soluble sugar and starch at late developmental stages. These genes are important in the manufacture of sugar and starch in vegetables. Real-time quantitative (RT-PCR) analysis was used to validate RNA-Seq data for 30 randomly chosen genes that appear to be helpful in garden pea breeding. In addition to a large number of differentially expressed genes (DEGs) connected to carotenoid biosynthesis, plant hormone pathways, sugar and cell wall metabolism during fruit ripening in watermelon, Zhu *et al*. (2017) [39] also discovered 797 novel genes to expand the reference gene set that is currently available. Pereira *et al*. (2018) [19] used a RIL population and a GBS-based genetic map to identify 33 QTLs affecting fruit quality parameters such as sugar and carotenoid content, fruit and seed morphology, and key loci controlling exterior colour of immature fruit and mottled rind in melon. Understanding evolutionary relationships was also made easier with the use of transcriptomic analysis. To date, the genomes of about 40 vegetables or closely related species have been sequenced, which points to rapid advancement in the use of genomic tools in breeding vegetable crops. These sequences obtained by NGS are typically deposited in the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/unigene).

3.2 Genome Wide Association Study (GWAS)

Whole genome association studies, or GWAS, look at genetic variations across the entire genome in a variety of people to determine whether any variation is linked to a particular trait. Single-nucleotide polymorphism (SNP) relationships with characteristics are the main focus of GWAS. A useful method

for determining the genetic basis of phenotypic variation is the development of the GWAS. It has been applied to comprehend the relationship between genetic variation and the intended attribute. To identify the loci governing variation in fruit metabolites, Sauvage *et al*. (2014) [23] used a core collection of 163 tomato accessions made up of *S. lycopersicum, S. lycopersicum* var. *cerasiforme*, and *Solanum pimpinellifolium*. They identified 44 loci in all, of which 19 characteristics, including levels of sucrose, ascorbate, malate, and citrate, were significantly related with. The draught genome sequence also opens up new areas for molecular studies. The GWAS was also shown to be beneficial in examining the evolution of several fruit quality attributes (fruit form, sweetness, and flesh colour) in watermelons (Gou *et al*., 2013). In a study of genetic diversity and unique genome-wide associations in capsicum, Colonna *et al*. (2019) [3] discovered four novel loci connected to the phenotypes regulating the shape of the fruit, including a non-synonymous mutation in the gene Longifolia 1-like (CA03g16080). Zhao *et al*. (2019) [38] conducted GWAS on tomato utilising 775 tomato accessions found 305 significant relationships for sugars, acids, amino acids, and volatiles associated with flavour. Thorwarth *et al*. (2018) [32] used 174 accessions to investigate the potential of GWAS and genetic prediction for enhancing curd-related features in cauliflower and found 24 significant relationships. Both increasing fruit size and accumulated capsaicinoid concentration are crucial quality characteristics of Capsicum annuum. Using GBS, Nimmakayala *et al*. (2016) [17] generated 66,960 SNPs and reported SNPs in Ankyrin-like protein, IKI3 family protein, ABC transporter G family, and pentatricopeptide repeat protein as markers for capsaicinoids. They also identified genomic sections linked to various fruit traits and capsaicin concentration in *C. annuum*.

3.3 SNPs discovery and use in genotyping platforms

Plant breeders can use the NGS to find genetic differences even with little technical knowledge and at a low cost. The accurate mining of genomic sequences for genetic variants that can be transformed into durable genetic markers, such as simple sequence repeats (SSRs) and single nucleotide polymorphism, was accelerated by parallel development of computational pipeline tools (SNPs). SSRs and SNPs are currently the most common and widely utilised markers in plant genetic study. The SNPs have evolved into preferred markers in contemporary breeding programmes because of their prevalence, stability, automation-friendliness, and affordability. Numerous vegetable crops, including the tomato for fruit metabolic properties, the muskmelon for fruit traits, the lettuce for moderate rates of post-harvest deterioration, the carrot for carotenoid biosynthesis genes, and the chilli for capsaicinoids, have SNPs (Nimmakayala *et al*. 2016) [17]. Generating genome-wide markers is another use for genotyping-by-sequencing (GBS). It is one of several low-cost, high-throughput genotyping approaches based on sequencing that effectively makes use of a fairly simple library building procedure and has been used to map QTLs in pumpkin (Zhang *et al*. 2015) [37].

3.4 Transgenic approach

Genetically modified (GM) or transgenic crops allow plant breeders to introduce advantageous genes into elite cultivars that were previously inaccessible, thus increasing the value of those cultivars. With the advancement of genetics and molecular biology, numerous quality-related genes have been discovered in various vegetable crops. These genes include those involved in pigmentation, the biosynthesis of vitamins, minerals, and flavour compounds, soluble carbohydrate metabolism etc. Vegetable breeders were able to add desirable transgenes to superior cultivars thanks to genetic engineering, increasing their worth, nutritional content, and other health advantages (Singh *et al*., 2020) [28]. Three genes from Erwinia have been inserted into potato to synthesise beta carotene: phytoene synthase (CrtB), phytoene desaturase (CrtI), and lycopene beta-cyclase (CrtY). By utilising a bacterial carotenoid gene (crtI) expressing the enzyme phytoene desaturase, transgenic tomato lines with increased carotenoid content were created. Gerjets and Sandmann (2006) created a genetically altered potato for keto-carotenoids (such as astaxanthin). According to Lu *et al*. (2006), transgenic cauliflower with or transgenesis may be linked to a biological mechanism that causes proplastids or other non-colored plastids to differentiate into chromoplasts in order to accumulate carotenoids. The or gene was also suggested to be a unique genetic strategy for inducing carotenoid accumulation in a significant staple food crop. Post-transcriptional gene silencing is also utilized to alter the pathways employed for nutrient production, improving the nutritional value. Tomatoes have undergone modifications in an effort to increase their flavour or nutritional value. Genes from the snapdragon plant (Antirrhinum) were employed to boost tomato anthocyanin levels (Tohge *et al*. 2015) [33]. Recent research has demonstrated that the RNAi-mediated inhibition of DET1 expression under fruit-specific promoters increases carotenoid and flavonoid levels in tomato fruits with little effects on plant growth (Williams *et al*. 2004) [34]. Diaz de la Garza *et al*. (2004, 2007) [5, 4] engineered tomatoes by over-expressing GTP cyclohydrolase I, which catalyses the first step of pteridine synthesis, and amino deoxychorismate synthase, which catalyses the first step of PABA synthesis, specifically in the fruit. This produced ripe tomato fruits that had an average of 25 times more folate than controls. It is possible to make comparable efforts to increase the folate content of other vegetables. Transgenic tomatoes with high amounts of folate and transgenic lettuce with increased tocopherol and resveratrol composition are promising discoveries against inadequacies (Dias and Ortiz, 2012). Additionally, transgenic cassava cultivars free of cyanide may be a potential approach for producing safe cassava (Siritunga and Sayre, 2003) [30]. Romer *et al*. (2000) [20] created a transgenic tomato to improve the carotenoid profile and content in tomato fruit. As a result, the amount of -carotene in cultivar "Ailsa Cray" has increased by about three times, reaching up to 45% of the total amount of carotenoids. According to research by Park *et al*. (2009) [18], lettuce that had the deregulated Arabidopsis H+/Ca2+ transporter sCAX1 (cation exchanger 1) had 25–32% more calcium than normal lettuce.

3.5 RNA interference

Crop development offers new avenues aided in the development of RNA interference (RNAi) and its regulatory potentials. In addition to having higher chances of product acceptance in the marketplace, RNAi technology is more precise, effective, stable, and superior to antisense technology. The novel gene-regulatory technique of RNA

silencing controls the amount of transcripts by either inhibiting transcription (TGS) or by inducing sequence-specific RNA degradation (PTGS/RNA interference). By increasing the antioxidant content of tomatoes, this technology has been successfully used to change the gene expression for improved quality traits. The significance of RNAi technology in prolonging tomato shelf life by preventing ACC oxidase gene expression (Xiong *et al*. 2005) [36]. Recent research has revealed that RNAi-mediated DET1 expression reduction under fruit-specific promoters increases carotenoid and flavonoid levels in tomato fruits with no effects on plant growth (Williams *et al*., 2004) [34]. In India, there have been scant attempts to use this revolutionary technique to increase vegetable harvests in carrot, tomatoes with high lycopene content, watermelons, and sweet peppers in particular. The RNAi technology, however, holds great promise for improving vegetable crops for specific traits like beta-carotene in tropical carrots and late bolting in palak, radish, and cauliflower, among others. It also holds great promise for reducing disease, insect pests, and male sterility for the production of hybrid seeds.

3.6 Targeted genome editing: Alternative trait-improvement technology for horticulture crops is targeted genome editing (TGE) technology. It is a new and innovative plant breeding method that has a higher mutagenesis efficiency than traditional breeding. Utilizing engineered nucleases such as meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 nucleases, the TGE enables targeted and stable editing of DNA. To create unique nucleases that target the desired DNA sequence, extensive engineering procedures are needed. These technologies relies on the binding and recognition of the nucleases to specific DNA sequences. Instead of using an endonuclease, CRISPR/Cas9 relies on a clear and inexpensive single guide RNA (sgRNA) that is complementary to the target sequence. Genome editing resulted in a 5.1-fold increase in the amount of lycopene in tomato fruit, according to Li *et al*. (2018) [12]. CRISPR/Cas9 has also been used to modify several anthocyanin and carotenoid biosynthesis pathway genes in tomato, such as Anthocyanin 1 (ANT1), Phytoene desaturase (SlPDS), Phytochrome interacting factor (SlPIF4), and Phytoene synthase (PSY1) (Hayut *et al*., 2017) [9].

Table 1: Status of RNAi and transgenic achievements in improving nutraceuticals

RNAi for nutraceuticals improvement in vegetable crops		
Tomato	*DET-1*	Enhanced flavonoids and carotenoid content.
	SlNCED-1(9-cis-epoxycarotenoid dioxygenase)	Enhanced lycopene and β-carotene content.
Transgenic achievements in improving nutraceuticals		
Tomato	Psy-1, Dxs, CrtB, CrtI, CrtY, CRY-2, CYC-B, LCY-B, LCY-B, CHY-B	Carotenoids-rich tomato
	Ros1 and Del.	Anthocyanin-rich tomato (Increased 70-100 folds higher than normal tomato)
	Petunia chi-a gene, CHI, CHS, CHI, F3H, FLS, MYB12, STS, CHR, FNS-II, Del, Ros1	Flavonols-rich tomato
	beta-Lcy gene	High β-carotene content
Cassava	cis-β-carotene	Enhanced β-carotene content

Table 2: Status of genome editing in improving nutraceuticals

Genome editing for nutraceuticals improvement in vegetable crops			
Tomato	Anthocyanin	*ANT-1*	CRISPR/Cas-9
	Carotenoids	*SIPDS*	CRISPR/Cas-9
	Carotenoids	*SIPIF4*	CRISPR/Cas-9
Watermelon	Carotenoid biosynthesis	Phytoene desaturase (*ClPDS*)	CRISPR/Cas-9
Tomato	β-carotene synthase	*Psy1*	TILLING

4. Conclusion

Nutritional value and related health advantages are major factors that are driving up their use among consumers. Despite having a high content of nutrients that are good for your health, some crops need improvement in order to increase the amount of these nutrients that the general population consumes. For this reason, the combination of genomics, transcriptomics, proteomics, and metabolomics aids in enhancing the nutraceutical content of vegetables. Genome-wide association studies (GWAS), phenotypic analysis through QTL mapping, genomic investigation, and other techniques are being used in conjunction with the recent advent of CRISPR/Cas9 genome editing in vegetable crops. Studies using microarrays and transcript profiling could identify the important physiological pathways. Studies on metabolism, its composition, and its role in numerous significant biological signalling cascades. By making use of numerous technologies that make it easier to detect relative alleles at loci in a single population, crop species genotype design has gotten simpler. This has therefore made it possible to produce better crop/plant varieties with improved nutraceutical qualities. Future possibilities for improving nutraceuticals through omics include lowering the cost of technology use, developing bioinformatics tools for data analysis and database storage, developing human resources for an overall scope of technology to apply in crop breeding, expanding the capacity of young scientists is required in breeding to manage, analyse, and interpret the vast data sets from omics, and establishing international relationships.

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