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Designing nutraceutical rich vegetable crops through conventional and molecular approaches

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Abstract

The compositions of ingested foods have relationships to positive but also adverse health effects, such as cardiovascular disorders, cancers, diabetes, hypertension and obesity. Vegetables are important components of the diet, supplying a multitude of health-related phytochemicals viz, vitamins (A, B₆, C, E, thiamine and niacin), phenolic compounds, carotenoids, alkaloids, flavanoids, minerals and dietary fiber. The current breeding trends addressed the feasibility of breaking down overall bioavailability into components such as anti nutrients and promoters. The *QTL1* and *QTL 2* of *Brassica villosa* is introgressed into *Brassica oleracea* var. *italica* for enhanced sulphoraphane. At AVRDC, the “Golden tomatoes” with 3 to 6 times higher ‘β’ than standard types were developed. Spontaneous mutation of semi-dominant “*Or*” gene enhanced β’ carotene in cauliflower curd. The two transcription factors, *Delila* and *Roseal* from *Antirrhinum majus* accumulate high amounts of anthocyanin in tomato fruits. The transgenic potato possessed enriched carotenoids a result of lipoprotein-carotenoid sequestering structures at chromoplast. To pace the breeding process, molecular-marker-assisted selection, transformation, chromosome manipulations and generation of useful mutant alleles have been employed.

Keywords: Vegetables, nutraceutical, health benefits, molecular marker

Introduction

Food habit has resulted in enhancing the nutraceutical value of vegetable crops which is a satisfying activity for plant breeders within the twenty first century. The term “Nutraceutical” is a combination of two words “Nutrition” and “Pharmaceutical” which was coined by DeFelice in the year 1989. It is defines as “A food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease”. In the present scenario, people are beginning to consume more healthful foods that can alleviate problems related to “diseases of overabundance” and diet-related chronic diseases, such as obesity, heart disease, and certain types of cancer. The chief immediate and long term objective of plant breeding is to enhance productivity to meet the ever increasing food requirement of people. Essential components, however, are quality attributes including nutraceuticals, colours and bioactive compounds etc. People are health conscious and use to take coloured vegetables rich in vitamins, minerals, antioxidants etc. And as a result of public perception that “natural is good”, increased global interest in nutraceuticals and edible colour consumption for their role in health protection. These characters are complex and determined by both genetical and environmental factors. Henceforth, identification and conservation of genetic resources of vegetable crops rich in colour and nutraceuticals is essential. The conventional breeding in conjunction with molecular biology will make it possible to get vegetable varieties enriched with nutraceuticals and edible colours suitable for fresh market as well as industry.

Bioactive Compounds**Carotenoids**

Carotenoids are a family of compounds of over 600 fat-soluble plant pigment that provide much of the visible colour in the food we eat. They are responsible for the color of tomatoes and carrots, and are partially responsible for fall coloration after the leaf chlorophyll has been destroyed. The most commonly studied carotenoids are β-carotene, lycopenes, lutein and zeaxanthin (Krinsky *et al*, 2005) [17]. Beta-Carotene and lycopene are hydrocarbons and belong to a class of carotenoids called carotenes that are fat-soluble and localized predominately in the low-density lipoproteins (LDL) in the circulation. Lutein and carotenoid belong to a category of carotenoids known as xanthophylls. Because xanthophylls are more polar than carotenes, therefore more evenly distributed among both LDL and high-density lipoprotein (HDL). (Clevidence and Bieri, 1993) [17]

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Anthocyanin

Anthocyanins are prompt as promising dietary compounds with a crucial role in human health. Anthocyanins (Greek anthos, flower and Greek kyanose, blue) are the largest group of water-soluble pigments in the plant kingdom and belong to the family of compounds known as flavonoids (Mazza *et al*, 2007) [24]. The red, purple and blue hues in fruits, vegetables, flowers and grains, however conjointly play necessary roles in plant physiology like attractants for insect pollinators and seed dispersal. Studies have demonstrated that anthocyanin extracts can improve sight acuteness (Ghosh *et al*, 2007) [12], display antioxidative and radical-scavenging activity (Astadi *et al*, 2009) [2] and to act as chemoprotective agents. Anthocyanins also play a role in anti-diabetic properties such as lipid lowering (DeFuria *et al*, 2009) [8], insulin secretion (Matsui *et al*, 2004) [23] and vasoprotective effects. (Matheus *et al*, 2006) [22]

Capsaicinoid and capsanthin

Capsaicinoids are health functional compounds that are produced uniquely in chilli pepper fruits placenta. The multiple capsaicinoid biosynthetic pathway genes (*Pun1*, *pAMT*, *KAS*, and *BCAT*) were strongly up-regulated in placental septum of pungent cultivars (Tanaka *et al*, 2017) [36]. Capsaicinoids (capsaicin and dihyrocapsaicin) have effective health benefit against rheumatoid arthritis, osteoarthritis, neuralgias, and diabetic neuropathy, weight management.

Glucosinolates

Glucosinolates (GSL) are sulfur- and nitrogen-containing secondary metabolites, well known for their role in inducing resistance against pathogens, maintaining auxin homeostasis in plants, and preventing cancer in humans (Woodward *et al*, 2005) [38]. GSLs are stable water-soluble compounds stored in the vacuoles of most of the plant's tissues. More than 120 GSLs have been reported so far, and most of them belong to the economically important crops of *Brassicaceae* family (Falk *et al*, 2004) [11]. Plants producing GSLs contain myrosinase (β -thioglucosidase) enzyme in specialized cells called myrosin cells (idioblasts) (Rask *et al*, 2000) [29]. When vegetables containing them are cut, glucosinolates convert to isothiocyanates (contain sulfur) and indoles (contain no sulfur). The isothiocyanates, dithiolthiones and sulforaphane are the bio-transformation products of glucosinolates that are involved in blocking enzymes which are responsible for tumorous growth in liver, lung, breast and gastrointestinal tracts (esophagus, abdomen and colon) (Baskar *et al*, 2012) [3]

Isoflavones

The subclass of phenols found in beans and other legumes like genistein and daidzein which effectively blocks tumor promoting enzymes. (Kaufman *et al*, 1997) [16]. Broad beans are enriched in phyto-nutrients such as isoflavone and plantsterols.

Lipoic acid

It can efficiently quench the hydroxyl radicals and is active both on lipids and tissue fluids. It protects catalase and glutathione, thus helpful in liver detoxification. Leafy green vegetables like spinach and broccoli have the highest concentrations of alpha-lipoic acid in the chloroplasts. The chloroplasts within the cells generated the energy or glucose in the broccoli. Other green vegetables (Peas, broccoli and

Brussels sprouts) also contain alpha-lipoic acid, though not in the concentrations found in leafy vegetables. (Drake, 2012) [9].

Nasunin

Anthocyanin first isolated from eggplant inhibits the hydroxyl radical generation (Nisha *et al*, 2009) [27]. The main natural supply of nasunin is found in the skin of eggplants (Brinjal) which is conjointly found within the purple radish, red turnip, and red cabbage. It provides the dark pigment in the fruit of the eggplant and also protects the eggplant from environmental damage especially from the sun and other radiant sources of energy. The major type of anthocyanin in purple brinjal is nasunin and has the high antioxidant activity.

Betalin

The phytochemicals found in beet and chart is usually utilised as natural colourant in ice creams due to its attractive deep colour. Moreover betalin is naturally absorbed by the body (Wettasinghe *et al*, 2002) [37]. It has anti radical scavenging activity (Cai *et al*, 2003) [5], anti-viral and anti microbial activity (Strack *et al*, 2003) [35]. Due to its nutraceutical properties it is being consumed in the form of food supplement "Betaxanthin" to fortify food product.

Charantia

Bitter gourd rank first among the cucurbits for higher nutritive value. This compound activates the inactive insulin present in the blood. Also have anti-HIV (MAP 30), anti-ulcer, anti-inflammatory, anti-leukemic, antimicrobial and anti-diabetic, and anti-tumor (Behera *et al*, 2007) [4]

Breeding Strategies

Existing genetic variation, trait heritability, gene action, associations among traits, and the availability of screening techniques and diagnostic tools are criteria commonly used to identify candidate traits and estimate potential genetic gains (Pfeiffer *et al*, 2007) [28]. When variation is not available, mutation and a transgenic approach may be the only remaining option (Al-Babili and Beyer, 2005) [1].

Breeding for enhanced action of nutrient

Consumption of broccoli (*Brassica oleracea* var. *italica*) is associated with a reduction in risk of prostate cancer (Joseph *et al*, 2004) [15], lung cancer (Spitz *et al*, 2000) [33] and colorectal adenomas (Lin *et al*, 1998). The anti-carcinogenic activity is most likely to be due to activity of the isothiocyanates **iberin** (1-isothiocyanato-3-methylsulfinylpropane, IB) and sulforaphane (1-isothiocyanato-4-methylsulphanylbutane, SF) derived, respectively from 3-methylsulphanylpropyl (3-MSP) and 4-methylsulphanylbutyl (4-MSB) glucosinolates that accumulate in the florets of broccoli. To enhance the levels of 4-methylsulphanylbutyl glucosinolate, the precursor of anti-carcinogenic isothiocyanate sulforaphane (SF), a segment of genome carrying *QTL1 (O2)* and *QTL 2 (O5)* is introgressed from the wild ancestor *Brassica villosa* (Faulkner *et al*, 1998) [10]. Allelic variations at two QTLs were of greatest potential importance in determining glucosinolate content. Variation at one of these QTL-1, affected both the total level and the ratio of 3-MSP and 4-MSB glucosinolates alone resulted in two to three fold increase in glucosinolate (Mithen *et al*, 2003) [30]. *B. villosa* alleles at QTL 2 could increase 3-MSP without a concomitant fall in 4-MSB. The two segregating populations

varied in their genotype at QTL-1, however QTL-1 and QTL-2 of *B. villosa* alleles interacted with each other to result in the expression of 3- MSP and 4-MSB (Sarikamis *et al*, 2006) [30].

Breeding for higher promoting factor

Promoters are necessary for the absorption of nutrients to the maximum. A small increase in these promoters may result in large improvement in bioavailability. For instance, ascorbate acts to stimulate transferrin-dependent iron uptake by an intracellular reductive mechanism thereby ascorbate-deficiency-induced anemia.

Breeding for reduced antinutritional factor

Faba beans (*Vicia faba* L.) have a great potential as a protein-rich crop, but anti-nutritional factor such as condensed tannins reduce the biological value of their protein. The seeds tannins can be removed by any of the two complementary genes, *zt-1* and *zt-2*, which also determine white-flowered plants. The less common factor, *zt-2*, is additionally related to accrued increased protein levels and energy values and reduced fibre content of the seeds. To identify a cost-effective method the (SCAR) marker linked to the *zt-2* gene have been identified. (Gutierrez *et al*, 2008) [13].

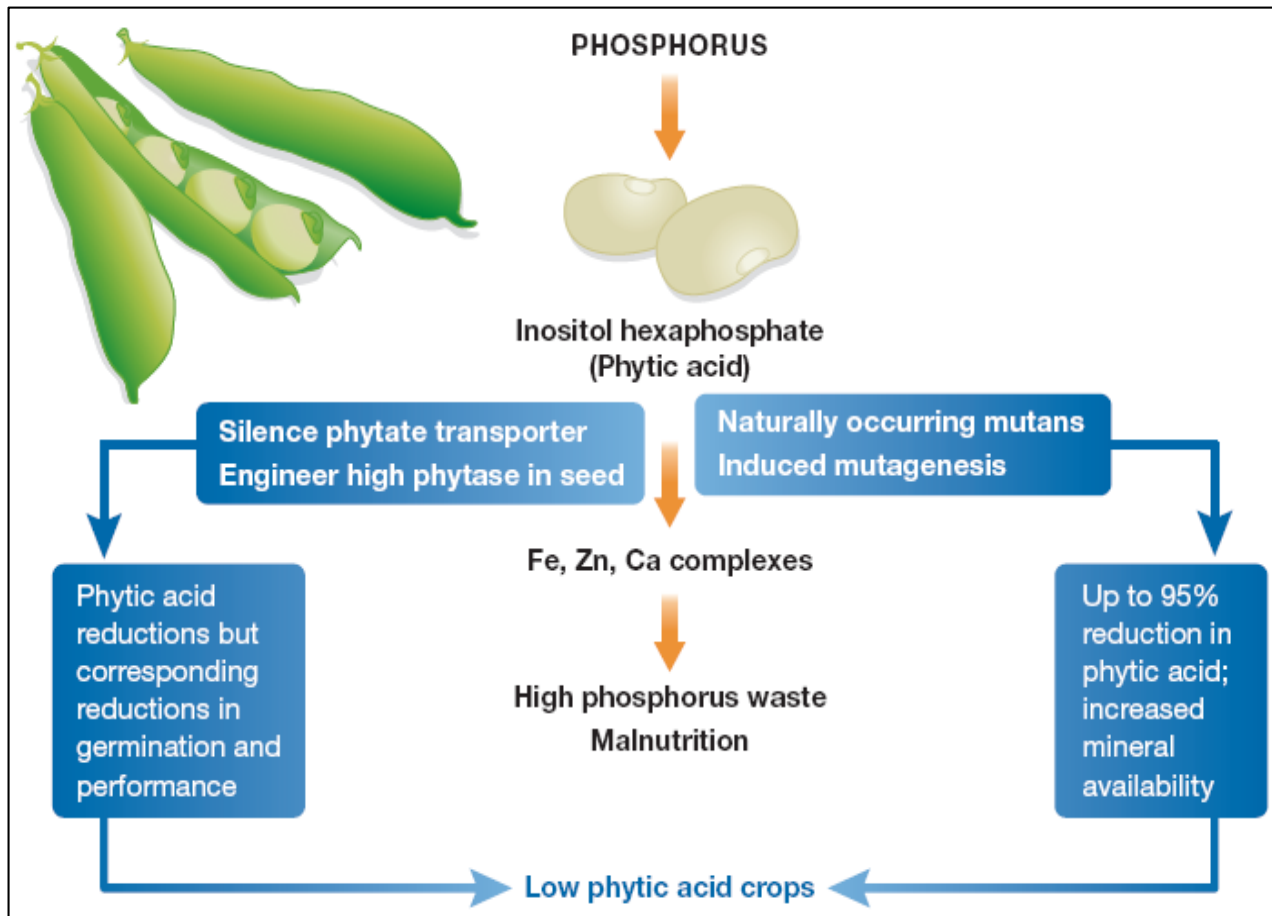


Fig 1: Approaches for reduced anti nutritional factor in legumes (Srinivas *et al*, 2017)

The common bean (*Phaseolus vulgaris* L.) genotypes were analysed for anti-nutritional factors *viz*, lectin, saponin, trypsin inhibitor and phytic acid. These toxins can cause neurological symptoms such as limb numbness, headache, chest tightness, and even severe coma, multiple organ damage or sudden death. The lectin, saponin, trypsin inhibitor and phytic acid in fresh pod were 1.743 mg g⁻¹, 3.730 mg g⁻¹, 1.680 mg g⁻¹, 3.102 mg g⁻¹ mg, respectively. The high-temperature cooking process deactivates but traces of endogenous toxins still remained (Shang *et al*. 2016). Henceforth, characterization of these compounds in several breeding lines will assist in the development of improved cultivar with minimum endogenous toxins for promoting food safety.

Breeding Approach

Pre-breeding

Inter-specific crosses

Introgression of alleles from *Solanum pimpinellifolium* to *Solanum lycopersicum* increase the β carotene content from

3.81- 6.55 mg/100 g FW compared to 0.60-0.9 mg/100 g FW for normal red-fruited tomato. The highest levels of lycopene, vitamin C, phenolics and solids contents were found in wild relative *S. pimpinellifolium* (Hanson *et al*, 2004). The dominant factor Anthocyanin fruit (*Aft*), which induces restricted pigmentation upon stimulation by high light intensity level, was introgressed into domesticated tomato plants by an interspecific cross with *S. chilense*, an *Abg* gene was introgressed from *Solanum lycopersicoides* which induce a strong and variegated pigmentation in the peel of tomatoes (Mes *et al*, 2008). A nutritious tomato line Golden tomatoes released by AVRDC in 2004 contain 3-6 times more vitamin A than standard types, a single tomato serves the daily Vitamin A requirement.

Mutation

An interesting associated distinctive Purple (*Pr*) gene mutation in cauliflower (*Brassica oleracea* var *botrytis*) confers an abnormal pattern of anthocyanin accumulation, giving the prominent mutant phenotype of intense purple

colour in curds and a few other tissues (Chiu *et al.*, 2010) [30]. The semi dominant *Or* gene induces many tissues of the plant, most noticeably the white edible curd and shoot apical meristem, to accumulate high levels of β -carotene, turning them orange. The β -carotene range from 3 to 320 $\mu\text{g}/100$ gram fresh tissue. The most interesting fact is that no alteration in carotenoid bio synthetic genes expression rather there the *Or* gene exert effect on lipoprotein carotenoid accumulation sink which is a novel mechanism (Li *et al.*, 2012) Plants that are heterozygous for *Or* possess bright orange coloration in these tissues and exhibit normal growth, while *Or* homozygous plants produce smaller curds with stunted growth, presumably due to unknown pleiotropic effects (Li *et al.*, 2003) [30]

Molecular Assisted Breeding

a) Identification of SCAR markers linked to *or* gene

The two RAPD markers—OPB01- 845 and OPAX18-656—and 1 AFLP marker- P67M54-172, to be linked to the *or* gene were identified by Zhang *et al.* (2008) in Chinese cabbage. The dominant markers were successfully converted to SCAR markers SCOR-845, SCOR204, and SCOR127, respectively. These SCAR markers were successfully mapped to linkage group with the *or* gene at a LOD score of 6.0. These SCOR127, SCOR204 and SCOR 845 were linked to *or* at distances of 10, 5, and 10 cM respectively on R9 or N9 of A genome linkage groups.

b) Identification of markers linked to *Pr* gene

The purple cauliflower were enriched with high anthocyanin compound which Chiu *et al.* (2010) [30] performed association mapping of 1,898 F2 individuals and identified molecular

markers BoMYB2, BoMYB3 and BoMYB4 from the on InDel and SNP region for the two alleles of the candidate *Pr* gene. The markers BoMYB2 and BoMYB4 were co- dominant while BoMYB3 was dominant.

c) Identification of markers linked to high GLS

A microsatellite marker OI12-F02 for QTL-2 were identified by Sarikamis *et al.* (2006) [30] in *Brassica. villosa* showing a 1:2:1 segregation of alleles for sulphoraphane. Hence, this marker can be efficiently employed for breeding of high desirable GLS in cauliflower.

Transgenic

The purple fruit colour of tomato was due to the expression of the two transcription factors, *Delila* and *Roseal* isolated from *Antirrhinum majus*. These transgenic tomato plants accumulating high amounts (70–100 fold) of anthocyanin during fruit ripening stages with maximum during the breaker stage (Maligeppagol *et al.*, 2013) [30]. For enhancing the carotenoid level in potato (*Solanum tuberosum*) Li *et al.* (2012) [30] from Cornell University developed transgenic potato tuber with enhances carotenoid accumulation. The provitamin A carotenoids in staple crops are not very stable during storage and their loss compromises nutritional quality. The transgenic potato tubers that expressed the cauliflower Orange (*Or*) gene is accountable for the formation of lipoprotein-carotenoid sequestering structures and increased abundance of phytoene synthase, a key enzyme in the carotenoid biosynthetic pathway. These have lead to the retention of β -carotene level and unceasingly stimulated its accumulation during cold storage.

Table 1: Important gene for nutraceuticals enhancement in vegetables (Singh *et al.*, 2015)

Vegetable	Nutrient enhancement	Gene
Tomato	Lycopene	High pigment (1,2,3) <i>hp</i> <i>ySAMdc;spe-2</i>
	Lycopene (Red)	Green ripe (<i>gr</i>)
	Lycopene (Reddish brown)	Green flesh (<i>gf</i>)
	β -carotene	Yellow (<i>r</i>)
		Tangerine (<i>t</i>)
		Betacarotene (<i>Beta</i>)
		<i>B</i>
	β -carotene (Yellow with reddish tinge)	Sherry (<i>sh</i>)
	β -carotene (Yellowish pink)	Apricot (<i>at</i>)
	β -carotene (Reddish orange)	Delta (<i>Del</i>)
	β -carotene (Dusky orange)	Diospyros (<i>dsp</i>)
	Lutein	<i>cry-2</i>
	Carotenoids	Phytoene synthase (<i>Psy-1</i>)
	Anthocyanin	Anthocyanin fruit (<i>Afi</i>)
		Aubergine (<i>Abg</i>)
Atroviolacium(<i>atv</i>)		
Tocopherol	<i>hmgr-1</i>	
Folate	<i>GCH-1</i>	
Kaempferol	<i>LC and C1</i>	
High flavonols	<i>chi-a</i>	
Chilli	Capsaicinoids	<i>C</i>
	β -carotene	<i>B; bc</i>
	Anthocyanin (Immature fruit)	<i>A/E</i>
Potato	Protein	<i>AmA1</i>
	β -carotene	<i>Or, Crt-B</i>
	Phytoene	<i>Dxs</i>
Sweet potato	High protein	<i>Asp-1</i>
	Anthocyanin	<i>IbMYB1</i>
Cauliflower	β -carotene	<i>Or</i>
	Anthocyanin	<i>Pr</i>

Cabbage	Anthocyanin	MYB
Lettuce	Folate	Gchl
	Iron	Pfe
	Ascorbate	Gul oxidase
Cucumber	β -carotene	Ore

Table 2: Nutraceutical rich vegetable varieties

Crop	Variety	Phytochemicals	Availability (Mg/100g Fw)
Carrot	Pusa Asita	Anthocyanin	339.29
	Pusa Rudhira	Lycopene	10.77
	Pusa Nayanjyoti	β -carotene	07.55
	Pusa Vrishti		3.35
	Pusa Yamdagni		4.60
	Pusa Meghali		4.77
	Arka Suraj		11.6
Radish	Pusa Jamuni	Anthocyanin	
	Pusa Gulabi	Lycopene	
Cauliflower	Pusa Betakesari	β carotene	0.80-1.00
	Arka Vimal	Antioxidant (DPPH)	213
		Vitamin	40
	Arka Spoorthi	Antioxidant (DPPH)	85.8
Vitamin C		49.3	
Red cabbage	Red Acre	Anthocyanin (Cyanidin)	
Bitter gourd	Pusa Aushadi	β carotene	
	Pusa Vishesh	Ca	420
		Fe	37.5
		Ca	350
	Pusa Hybrid -2	Fe	15.50
Ridge gourd	Arka Prasan	Antioxidant and minerals (P, Ca and Zn)	
	Arka Vikram	Antioxidant and minerals (P, Ca, Mn, Fe and Zn)	
Beet leaf/ Palak	Arka Anupama	Protein, carotenes, Vitamin C, and minerals (P & K)	
		Nitrate content	Less than the local variety
	Pusa Bharati	Ascorbic acid	
	Pusa Jyoti	Vitamin A,C, Fe, Ca	
Vegetable Mustard	Pusa Sag-1	β carotene & Vitamin C	
Chenopod	Pusa Bathua -1	Ascorbic acid	23.0
Spinach	PS -1	Ascorbic acid	51.0
Amaranthus	Pusa Lal Chaulai	Carotenoids	69.4
	Pusa Kiran		59.0
	Arka Suguna	Protein, carotene, vitamin C and minerals (P & K)	
		Antioxidant	499.00
		Nitrate	27.30
	Arka Samraksha	Oxalate	1340
		Leaf protein	4.0 %
		Antioxidant	
		Antioxidant	417 AEAC
	Arka Varna	Nitrate	37.6
Oxalate content / 100 g fr. wt.		1420	
Leaf protein		4.1 %	
Coriander	Arka Isha	Vitamin C	167.05
		Leaf essential oil	0.083%

Conclusion

It is imperative that the nutrients found in many foods, fruits and vegetables are responsible for the well documented health benefits. For example, lutein and zeaxanthin prevent cataracts and macular degeneration; beta-carotene and lycopene defend the skin from ultraviolet radiation damage; lutein and lycopene may benefit cardiovascular health, and lycopene might prevent prostate cancer. Because of the different marked health benefits of these, it must be taken regularly and to reduce the risk factors like high cholesterol, high blood pressure and diabetes. A great diversity of vegetables ought to

be eaten to make sure that individual's diet includes a combination of phytonutrients and to get all the health benefits. Regular consumption of a high vegetable diet has plain positive effects on health since phytonutrients of vegetables can defend the physique from many varieties of chronic diseases. Cruciferous vegetables, Allium sp, tomato, cucurbits, soybean, carrot, okra, underexploited vegetables like lettuce, coleus, sweet potato, yams, moringa, winged bean, basella, cluster bean etc. are good sources of bioactive compounds. In recent years, the growing demand of traditional vegetable is due to high nutritional content as well

as its hardiness and ability to complete its life cycle in short duration. To pace the breeding program, modern techniques *viz.*, the molecular genetics and modern biotechnology approaches in conjunction with deciphering the metabolome of a crop plant are powerful tools that will facilitate in specific redesigning of metabolism in food crops to accumulate desired, or close-to-the-desired, levels of a particular phytonutrient. Additional analysis is required in several areas to make sure this rising science continues to be valid and is translated speedily into consumer-relevant product. From breeder perspectives, exploring vegetables to their full potential is necessary for safeguarding human health; understanding the biosynthetic pathways and relationship between metabolites. Enhancing promoters and breaking down of antinutrients contents and finally enhancing the bioavailability that will reach to the target region have been the primitive goal.

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