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Advances in vegetable breeding for nutraceuticals and quality traits

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Abstract

Vegetable crops are rich in dietary micronutrients (minerals, vitamins), antioxidents and functional phytochemicals 'nutraceuticals'. However, some of the elements are of public health concern such as β-carotene, iron, calcium and folic acid which attracts breeders' interest to improve their content in food sources including vegetable crops so that their dietary intake could be increased and their deficiency problems reduced. Some nutraceutical compounds such as anthocyanin, β-carotene, lycopene and glucosinolates have been reported to play active role in health and their contents have been found more in beet root, cauliflower, carrot and broccoli, respectively. The attempts through conventional breeding methods have been slow and inadequate, hence new developments in molecular and biochemical diagnostics tools and techniques showed promise in improving the nutraceutical contents and other quality traits. Molecular markers, next generation sequencing, RNA interference (RNAi) and genetic engineering have great promise in reducing time and cost involved in quality breeding. The present review summarise attempts and potential of improving the nutraceuticals and quality traits in vegetable crops.

Key words: Vegetable crops, quality traits, nutraceuticals, molecular markers, breeding.

Introduction

Vegetables are important source of dietary minerals and vitamins and contribute to fight against micronutrient malnutrition which affects nearly 795 million people worldwide including 194.6 million in India (Stein and Qaim 2007). They contribute functionally active secondary metabolites which have preventive role against various health ailments, particularly non-communicable diseases. The health benefiting compounds in vegetables are vitamins, carotenoids, phenolics, alkaloids, flavonoids, nitrogen containing compounds, organo-sulphur compounds etc. These secondary metabolites act as antioxidants in human body and play role in homeostasis balance in body by reducing synthesis and/or squeezing the reactive free radicals. Due to functional role of these compounds in human health, they are considered natural nutraceuticals. Hence, vegetables rich in antioxidants have great potential in managing non-communicable diseases such as obesity, cardio-vascular disease, certain types of cancer, phytoestrogens, anti-inflammatory agents and nutritional deficiencies (Steinmetz and Potter 1996; Bliss 1999; Slavin and Lioyd 2012). Some of the investigations showed increased bioavailability of dietary minerals such as calcium from biofortified crops (Morris et al. 2008) however, there is need of enriching target micronutrients or reducing the anti-nutrients in vegetables to ensure higher bioavailability in vegetarian diets. Although, scientific evidences for extent of bioavailability of dietary minerals and antioxidants from vegetables in different demographic profile is not adequate (Patel and Srinivasan 2016) but intake of diets rich in diverse vegetables balances the nutrient requirement.

Globally, the Food and Agriculture Organization (FAO) recommends for intake of 400 g vegetables per day and in order to boost the consumption, the FAO and World Health Organization (WHO) have launched Promotion of Fruit and Vegetables for Health (PROFAV) as a global initiative. For Indian population, the Indian Council of Medical Research (Anonymous 2010) recommends 125 g leafy vegetables, 100 g root and tubers and 75 g other vegetables per capita per day. To attain the target, the vegetable production in the country has reached at 175 million MT with annual growth rate of 3%. Theoretically, the vegetables production indicates gross availability of 378.13 g per

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capita per day and net availability of vegetables (after deduction of 25% loss+ 5% exports and processing) is 286 g per capita per day (Anonymous 2017). Further, successful attempts in biofortification of vegetables such as carrot (for calcium), sweet potato, potato, cassava and cauliflower (β-carotene), potato (reduced amylose and increased amylopectin in starch granules), cassava (iron and protein) and lettuce (iron) (Garg et al. 2018) highlights the attention on vegetable crops for improvement programme for nutraceuticals and quality traits for betterment of public health.

Nutraceuticals in vegetables

Vegetables form an essential component of a balanced diet and provide a significant share of dietary demand of minerals and vitamins. Nutraceuticals are among the important constituents of quality attributes in vegetable crops (Fig. 1). Nutraceuticals and bioactive compounds found in different vegetable crops reported to have varied health benefits. Allyl sulfides in *Allium* vegetables (garlic, onions, chives, leeks) protect against certain cancers and heart diseases, boost the immune system; glucosinolates from Cole vegetables play a key role in protection against cancer, heart disease and stroke. The β-carotene is important for healthy skin and membranes, immune system, and good for eye health; lycopene from tomato, watermelon, carrot and red peppers fights against prostate cancer; ascorbic acid rich green peppers, broccoli, green leafy vegetables, cabbage and tomatoes is helpful in growth, development and repair of all body tissues and absorption of iron from foods. The momordicin and charantin from bitter gourd have anti-diabetic properties and help in blood purification. Most vegetables are naturally low in fat and calories while none have cholesterol, hence, good for heart. Dietary fibre reduces blood cholesterol levels, lowers risk of heart disease and improves bowel functioning.

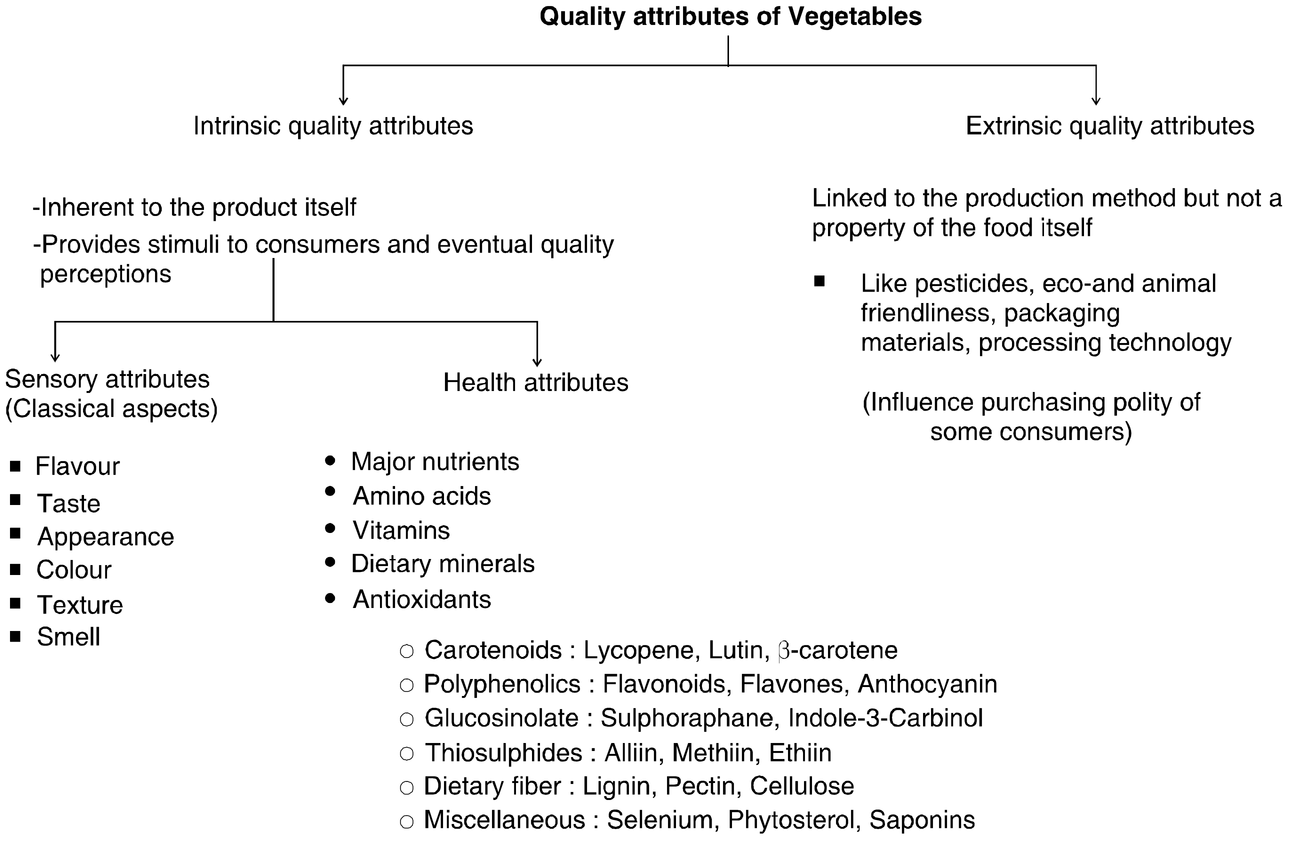

Fig. 1. Classification of quality traits in vegetable crops

India is bestowed with huge number of wild or cultivated vegetable crops which are rich in antioxidants and dietary minerals (Thongam et al. 2016; Singh et al. 2018). There is plenty of diversity within vegetable species such as in case of tomato for antioxidants and lycopene (Pillakenchappa et al. 2014; Bhandari et al. 2016), in chilli for capsaicin (Misra et al. 2011), watermelon for lycopene (Perkins-Veazie et al. 2006), glucosinolates in cauliflower and broccoli (Kushad et al. 1999). The biochemical pathway for synthesis of the antioxidant compounds are predominantly controlled by quantitative trait loci (QTLs) and influenced by environmental factors. The information on such QTLs is very less in case of vegetable crop germplasm and handling of such complex traits advance molecular and genomics tools and techniques.

Breeding objectives for quality vegetables

The vegetables are perishable food items and their regular supply in adequate quantity remains a big challenge. Hence, measures to enhance production of vegetables through home gardens, kitchen gardens, container gardening, roof gardening, etc. are quite attractive not only for remote rural areas but also urban people. Establishment of new home gardens or enriching the existing traditional home gardens with nutrient rich locally adaptable crops and their superior varieties could serve much better in term of nutritional security. This of course, requires identification of region specific vegetable crops and breeding of varieties rich in dietary nutrients, prolonged harvest period, adaptable to growing situation and high acceptance among the ultimate beneficiaries. The breeding objectives could be rich in dietary nutrients and antioxidants, good in organoleptic scores, tolerance to partial shade, high portion of edible fruits/ parts, low gestation period, tolerant to diseases and pest, responsive to organic sources etc. So far, research efforts remained targeted towards development of technologies and varieties for commercial scale but some varieties are quite fit for small scale growing in gardens. But, efforts are utmost required to develop plant types which are ideal for home gardens or other micro-scale production systems. In general, the vegetable breeding for nutrients is centred on high yield and rich in dietary micronutrients which are traditional traits but enrichment of nutraceuticals need special attention right from identification of breeding materials to handling the breeding programme and maintenance of the improved varieties.The futuristic traits are affected by consumer preferences and changes with time scale. Nutraceutical traits varies with crop such as for carrot, it is required to breed varieties rich in β-carotene, lycopene and anthocyanin stable at higher temperatures, high proportion of bioavailable forms of micronutrients (Ca, Fe, Zn) and target carotenoids, mild fragrance. The important quality traits of cole crops are β-carotene, glucosinolate, anthocyanin, iron and zinc, their stability in different growing temperature regimes, better retention of bioactive compounds during post-harvest life and mild flavour. High lycopene stable at high temperature, TSS and strong antioxidant activity in tomato are preferred traits in varieties rich in minerals and vitamins, low in anti-nutrients and high bioavailability of micronutrients are required in leafy vegetables. High level of bioavailable form of proteins and low anti-nutrients (trypsin inhibitor) are targets for legume vegetables; cucurbits rich in functional constituents in fruits (cucurbitacins, momordicins, carotenoids minerals etc.), leaves (minerals, vitamin) and seeds (essential fatty acids) are target traits.

Requirement for breeding antioxidant and quality rich vegetables

Although, no systematic programme has been taken up for breeding biofortified varieties in vegetable crops but there are varieties/hybrids developed by conventional breeding in different vegetable crops which are rich in antioxidants and colour compounds (Table 1). However, rising demand of biofortified crops warrants to provide nutrient enriched varieties at a rapid pace to meet the market demand. For this, a combination of robust genetic source, modern genomics tools and biochemical diagnostic techniques is must for success of quality breeding programme.

The pre-requisites for breeding nutrient rich varieties are: (i) high nutrient density must be combined with high yields and high profitability; (ii) the micronutrient status of human subjects must be shown to improve when consuming the biofortified varieties as normally eaten; (iii) sufficient nutrients must be retained in processing and cooking and these nutrients must be sufficiently bioavailable; and (iv) the enriched/ biofortified varieties must be adopted by farmers and consumed by those suffering from micronutrient malnutrition in significant numbers. Further, for breeding nutrient rich varieties in a crop, the exploration of available genetic resources is required for: (i) parental genotypes that can be used in crosses, genetic studies, molecular marker development, and (ii) existing varieties, pre-varieties in the release pipeline or finished germplasm products for fast tracking.

Pre-breeding for quality traits in vegetable crops

Pre-breeding encompasses activities of: (i) identification of potential donor sources for trait(s) of importance in wild species or wild crop relatives, (ii) introgressing them into crop genetic backgrounds to

Table 1. Common nutraceutical compounds in vegetable crops and their role in plant and human health

Nutraceuticals	Role in plant system	Role in human health	Vegetable crops and varieties rich in neutraceuticals
Phenolics	Signaling molecules, pigments, flavor, defense	Antioxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic, reduce cardiovascular diseases	Brinjal (Pusa Safed Baingan 1, 31.21 mg/100 g fw; Pusa Hara Baingan 1, 33.5 mg/100 g fw), Bitter gourd (Pusa Aushadhi, 6.51 mg/100 g fw; Pusa Rasdhar (4.3 mg/100 g fw), Onion (Quercetin: Pusa Madhvi, 101.2 mg; Pusa Ridhi, 107.42 mg; Pusa Soumya, 74.6 mg/100g fw)
Carotenoids	Pigmentation, attract pollinators	Anticancer, anti-cardiovascular, eye health, anti-oxidant, prostate cancer	Beta-carotene: Carrot (Pusa Yamdagini, 7.55 mg/100g fw), Pusa Nayanjyoti, Nantes, Pusa Meghali (tropical type), Muskmelon (1.15 - 12.82 mg/ kg, Henan et al. (2013) Lycopene: Watermelon (23-72 ìg/g fw; Rao and Rao, 2007), Carrot (Pusa Rudhira, 386 mg/100g fw),Tomato (Pusa Rohini 4.5 mg/100g fw)
Anthocyanin	Pigmentation (purple, red)	Antioxidant, anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties	Root crops: Carrot (Pusa Asita, 339 mg/100g fw), Beet root (Crosby, 17.15 mg/g dm; Sawicki et al. 2016), Cole crops**:** Red cabbage (Primero 109 mg/100g fw, Ahmadian et al. 2014), Purple Cauliflower (Graffiti, 375 mg/100g fw, Chiu et al. 2010); Purple Broccoli (48 mg/100 g fw), Brinjal (Pusa Shyamla, 48.2 mg), Red Amaranth (Pusa Lal Chaulai)
Glucosinolates	Plant defence	Anti-cancer compounds	Broccoli (Green broccoli, 15.2-59.3 µ mol/100 g fw), Purple Broccoli (26.3 µ mol/100 g fw), Purple cauliflower (35.69 µ mol/100 g fw), Cauliflower (19.5-42.6 µ mol/100 g fw; sinigrin in Pusa Sharad 23.94 µ mol/100 g fw), Red cabbage (30.1-98.3 µ mol/100 g fw), Brussels sprouts (16.6-36.9 µ mol/100 g fw) (Verkerk et al. 2009)
Dietary minerals:	Proper growth and development	Essential for health such as Iron (Fe) for hemoglobin Calcium (Ca) for bones	Iron: Bathua (Pusa Green, 7.6 mg/100g dw), Palak (All Green, 16.2 mg/100g dw), Amaranth (Pusa Kirti (38.5 mg/100g dw), Methi (Pusa Early Bunching, 17.2 mg/100g dw), Sag sarson (PusaSag -1, 16.3 mg/100g dw) Calcium: Amaranth (330-800 mg/100g dw), palak (380 mg), knol-khol (368 mg/100g dw)

develop genetic stocks, and (iii) minimize linkage drag. Molecular markers for both fore ground and background selections are of great importance to increase efficiency of pre-breedingby reducing time and cost involved. In vegetable crops, pre-breeding has been successfully employed for developing health beneficial glucosinolates (3-methylsulphinylpropyl or 4-methylsulphinylbutyl) rich genetic stocks in broccoli by marker assisted introgression of QTL1 and QTL2 from *Brassica villosa* (Sarikamis et al. 2006). Leiva-Brondo et al. (2016) identified, accessions BGV6195 of *Solanum pimpinellifolium*, LA1423 of *S. Lycopersicon* var. *cerasiforme* and LA3633, a possible hybrid between *S. pimpinellifolium* and *S. lycopersicon* var. *cerasiforme* with high genotypic potential for the improvement of lycopene content in tomato.

Breeding methods for quality traits

The breeding for nutrient enhancement in vegetable crops is done through combining one of more micronutrients from germplasm using traditional and or advance breeding methods. Use of molecular markers for identification and transfer of gene(s) and QTLs for nutrients and their use in biofortification is now frequent in vegetable crops. Nutrient levels in crop plants are under genetic control hence, the prospects for improving them through breeding are enormous. However, its success depends upon the preciseness of genetic architecture and effective breeding system. The choice of breeding method would be largely guided by the nature of gene action and relative magnitude of additive genetic variance, dominance variance and epistasis in a breeding population. The efficient breeding procedure should be effective in manipulation and selection of favourable gene combination, additive genetic variance, exploitation of dominance variance and achieving close relationship between expected genetic gain and realized progress by selection. However, it is important to have knowledge of existing genetic variation, trait heritability, gene action, association among the traits, available screening techniques and diagnostic tools.

Advanced techniques in vegetable breeding

Mutation breeding

Out of 3000 mutant varieties developed globally, 776 have been induced for nutritional quality (Jain and Suprasanna 2007). In a simple way, mutation is a random or directed change in the structure of DNA or the chromosome which often result in a visible or detectable chance in specific trait. Relatively minor genomic changes (point mutations, single gene insertions) are regularly observed following metabolomic analysis, leading to significant changes in biochemical composition and antioxidants (anthocyanin, lycopene) in the tomato cv. Moneymaker (Giliberto et al. 2005). Sapir et al. (2008) reported in tomato that *high pigment-1* (*hp-1*) mutation known to increase flavonoids content in fruits. Spontaneous mutation such as 'orange' cauliflower (Li et al. 2001) and in sweet potato orange mutants are rich in beta-carotene (30-100 ppm) than white fleshed (2 ppm) and some of the common orange fleshed mutant varieties are Nancy Gold and Murff Bush Porto Rico (LaBonte and Don 2012). Further, development of Targeting Induced Local Lesions in Genomes (TILLING) (Till et al. 2003) opened new era to identify all allelic variants of a DNA region present in a collection of artificial mutant. Similarly, EcoTILLING also is an option to identify allelic variants for targeting genes in natural collections (Comai et al. 2004). Mianoia et al. (2010) used TILLING technology to develop new mutant genetic resource for tomato crop improvement.

Polyploidy breeding

Polyploid can be induced due to aberration in cell division. This may occur both in the mitosis as well as in meiosis. This method can be used successfully in vegetable breeding as a means of enhancing nutraceuticals and colours. Tetraploids in radish, pumpkin, muskmelon and watermelon are highly productive and have improved quality. Zhang et al. (2010) developed tetraploid muskmelon which is rich in soluble solid, soluble sugar and vitamin C contents and distinctly higher than those in the diploid fruit. Liu et al. (2010) reported that the range of lycopene content among diploid watermelon fruit was from 33.2 to 54.8 mg/kg whereas in triploid it was from 41.2 to 61.8 mg/kg. The range of lycopene content among tetraploid was from 38.1 to 59.8 mg/kg. They also reported that the lycopene content in the triploid and tetraploid was higher than that of diploid, however ploidy did not affect lycopene content in variety 'Fan Zu No.2'.Marzougui et al. (2009) induced polyploidy in *Trigonella foenum-graecum* L. using a 0.5% colchicine solution and reported that the autotetraploid cultivar has larger leaf area and larger productivity compared to the diploids concerning seed number, pod number and branch number. Its leaves are rich in potassium, sodium, calcium and phosphorus.

Molecular breeding for nutrient enrichment

Molecular markers such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), microsatellites or simple sequence repeats (SSR), sequence characterized regions (SCAR), cleaved amplified polymorphic sequences (CAPS), sequence tag sites (STSs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs) and diversity arrays technology (DArT) are used to study linkage with gene responsible for high nutraceuticals and edible colours using mapping population. In MAS a marker (morphological, biochemical or one based on DNA/RNA variation) is used for indirect selection of a trait of interest. The mapping populations such as Near Isogenic Lines (NILs) and Recombinant Inbreed Lines (RILs) are used to identify the molecular marker linked to genes. Zhang et al. (2008) found SCAR markers linked to "*or*" gene inducing β-carotene accumulation in Chinese cabbage. Ripley and Roslinsky (2005) identified an ISSR Marker for 2-propenyl glucosinolate content in *Brassica*. The orange cauliflower (first discovered in Bradford Marsh, Ontario, Canada in 1970) results from a spontaneous mutation of a single dominant gene designated as '*Or*' for orange gene (Dickson et al. 1988). This *Or* mutant was originally found in white curded autumn crop cv. Extra Early Snowball. This trait is absent in Indian cauliflower where large population is suffering from carotene deficiency. Kalia et al. (2018) at IARI designed and orient biofortification of Indian cauliflower with âcarotene enhancing native '*Or*' gene following marker assisted breeding.

Genome sequencing and transcriptome analysis for quality traits in vegetables

The NGS technologies have reduced the cost and time for sequencing compared to Sanger technology and making it possible to perform millions of sequencing reactions in parallel. Second generation sequencing (massively-paralleled sequencing) technologies such as Roche/454 pyrosequencing and Illumina/Solexa sequencing are already profusely used to sequence plant species and third-generation sequencing (also known as long-read sequencing) are currently under active development and incorporated to sequencing projects PacBio RS (Pacific Biosciences), Helicos (Helicos), or Ion Torrent (Life Technologies). Nowadays, it is feasible to sequence most crop genomes (excluding those with a very large and complex genome) with a relatively modest budget, by combining Sanger with next generation sequencing technologies. However, as comparative to whole genome sequencing transcriptome sequencing has been a cheaper alternative. The RNA-seq is independent of a priori knowledge on the sequence under investigation, also allowing thereby analysis of poorly characterized species. It helps to study gene expression and identify novel RNA species and directly reveals sequence identity, crucial for analysis of unknown genes and novel transcript isoforms. Transcriptomic analysis has been used in a number of vegetable crops for understanding the quality related traits. Zhu et al. (2017) analysed transcriptomic changes occurring during the browning of fresh-cut fruits from *Luffa* cultivar 'Fusi-3' and identified 11 genes from five gene families (i.e., *PPO*, *PAL*, *POD*, *CAT*, and *SOD*) as potentially associated with enzymatic browning as well as four WRKY transcription factors (key components in abscisic acid signalling) observed to be differentially regulated in fresh-cut *Luffa* fruits. Mutant alleles at three loci *r* (referred to ADP-glucose pyrophosphorylase), *rb* (starch branching enzyme), and *bsg* (phosphoglucomutase) in pea affect starch and sugar synthesis (Harrison et al. 2000). The presence of *r* or *rb* or *bsg* genes results into accumulation of low level of starch and a high level of sucrose. Recently, Liu et al. (2015) performed a comparative transcroptomic analysis of genes involved in sugar and starch biosynthesis in vegetables and reported differential expression of genes related soluble sugar and starch at late development stages. Validation of RNA-Seq data was done using real-time quantitative (RT-PCR) analysis for 30 randomly selected genes which seems to be useful in garden pea breeding. Zhu et al. (2017) identified 797 novel genes to extend the available reference gene set and a large number of differentially expressed genes (DEGs) related to carotenoid biosynthesis, plant hormone pathways, and sugar and cell wall metabolism during fruit ripening in watermelon. Transcriptomic analysis also helped in understanding of phylogenetic relationship. Using transcriptome sequencing data, Xu et al. (2015) identified ~320,000 high-quality SNPs in spinach and indicated that *Spinacia turkestanica* was more closely related to the cultivated *S. oleracea* than *S. tetrandra*. Such information will be useful for exploring wild resources for quality improvement of vegetables. These sequences obtained by NGS are generally deposited in the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/unigene) and till date genomes of around 40 vegetables or related species have been sequenced which indicates for rapid progress in use of genomic tools in breeding vegetable crops.

SNPs discovery and use in genotyping platforms

The NGS is very helpful for plant breeders to discover genetic variation even with limited technical expertise and at minimal cost. Parallel development of computational pipeline tools accelerated accurate mining of genomic sequences for genetic variants that can be converted into robust genetic markers such as simple sequence repeats (SSRs) and single nucleotide polymorphism (SNPs) (Deschamps and Campbell 2010). Now-a-days, the SSRs and SNPs are the predominant and mostly used markers in plant genetic analysis. The SNPs have become choice markers in modern breeding programme due to their abundance, stability, amenability to automation, and cost-effectiveness (Ganal et al. 2009). SNPs have been discovered in a number of vegetable crop such as tomato for fruit metabolic traits (Viquez-Zamora et al. 2013), muskmelon for fruit traits (Chang et al. 2016), lettuce for slow rates of post-harvest deterioration (Simko et al. 2018), carrot for carotenoid biosynthesis genes (Jourdan et al. 2015) and chilli for capsaicinoids (Nimmakayala et al. 2016). The genotyping-by-sequencing (GBS) is also in use for generating genome-wide markers. It is one of a low cost and high-throughput sequencing-based genotyping approach that efficiently utilizes a relatively simple library construction protocol (Elshire et al. 2011) and have been used in mapping QTLs in pumpkin (Zhang et al. 2015).

Transgenic approach for quality improvement

Three genes, namely, encoding phytoene synthase (*CrtB*), phytoene desaturase (*CrtI*) and lycopene beta-cyclase (*CrtY*) from *Erwinia* have been introduced in potato to produce beta carotene. Transgenic tomato lines were developed with enhanced carotenoid content by using a bacterial carotenoid gene (*crtI*) encoding the enzyme phytoene desaturase. Genetically engineered potato for keto-carotenoids (including astaxanthin) was developed by Gerjets and Sandmann (2006). Lu et al. (2006) suggested that transgenic cauliflower with *Or* transgenesis associated with a cellular process that triggers the differentiation of proplastidsor other non-coloured plastids into chromoplasts for carotenoids accumulation. They also indicated that the *Or* gene can be used as a novel genetic tool to induce carotenoid accumulation in a major staple food crop. The post-transcriptional gene silencing is also used for change in nutrient biosynthesis pathways which improve the nutrient contents. Tomatoes have been altered in attempts to improve their flavour or nutritional content. The snapdragon (*Antirrhinum*) genes were used to increase anthocyanin concentrations in tomato (Tohge et al. 2015). The RNAi-mediated suppression of DET1 expression under fruit-specific promoters has been recently shown to improve carotenoid and flavonoid levels in tomato fruits with minimal effects on plant growth (Williams et al.2004). Folate deficiency is serious issue in public and Diaz de la Garza et al. (2004, 2007) engineered tomatoes by fruit- specific over-expression of GTP cyclohydrolase I (catalyzes first step of pteridine synthesis) and amino deoxychorismate synthase (catalyze the first step of PABA synthesis) which resulted into ripe tomato fruits with an average 25-fold more folate than controls. Similar attempts can be tried to enrich folate content in other vegetables.

RNA interference in vegetables for quality traits

The discovery of RNA interference (RNAi) and its regulatory potentials has opened new vista for crop improvement (Jagtap et al. 2011). RNAi technology is precise, efficient, stable and better than antisense technology and even the chances of acceptance of products is more in public. The RNA silencing 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) (Agrawal et al. 2003).This technology has been demployed successfully to alter the gene expressions for improved quality traits by increasing antioxidants in tomatoes (Niggeweg et al. 2004) or suppressing over expression of negative traits such as sinapate esters in canola (Husken et al. 2005) and alpha-linolenic acid in soybean (Flores et al. 2008). The role of RNAi technology in extending the shelf-life of tomato by blocking the expression of ACC oxidase gene (Xiong et al. 2005) and suppression of two ripening specific N-glycoprotein modifying enzymes, α-mannosidase and β-D-*N*-acytlhexosaminidase (β-Hex) (Meli et al. 2010) is well established. Peters et al. (2011) used RNAi technology to develop Dau c 1.01 and Dau c 1.02-silenced transgenic carrot plants show reduced allergenicity to patients with carrot allergy. McCormick et al. (2004) developed virus (*Carrot virus Y*, CarVY) resistant carrot genotypes using RNAi technology. Moreno et al. (2013) also demonstrated that a pronounced reduction in storage root thickness and colour of carrots was obtained in *DcLcyb1* transgenic silenced lines. Negligible attempts were made to utilize this novel technology for improvement of vegetable crops in general and carrot, high lycopane in tomato, watermelon and sweet pepper in particular in India. However, the RNAi technology has great promise in improvement of vegetable crops for specific traits like beta-carotene in tropical carrot and late bolting in palak, radish and cauliflower etc. diseases and insect pests and male sterility for hybrid seed production.

Targeted genome editing

Subburaj et al. (2016) highlighted targeted genome editing (TGE) technology as an alternative tool for trait improvement in horticultural crops. It is an emerging and novel plant-breeding tool which is alternative to classical breeding having higher mutagenic efficiency. The TGE facilitates targeted and stable editing of DNA using engineered nucleases including meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)/ Cas9 nucleases. Meganucleases, ZFNs, and TALENs depend on the binding and recognition of the nucleases to specific sequences of DNA hence, complex engineering processes are required to produce custom nucleases that target the sequence of interest (Martin-Pizarro and Pose 2018). The CRISPR/Cas9 does not depend on the endonuclease, but on a simple and cheap design of a single guide RNA (sgRNA) that is complementary to the target sequence (Jinek et al. 2012). Martin-Pizarro and Pose (2018) has exclusively reviewed use of genome editing tools for fruit ripening manipulation. Li et al. (2018) reported about 5.1-fold increase in lycopene content in tomato fruit through genome editing.

Conclusion

Nutritional qualities and associated health benefits of vegetable crops is becoming important criteria for their increase in consumers diet. Although vegetables are rich in health beneficial compounds but in certain crops the content of public health significance nutrients (β-carotene ascorbic acid, iron, calcium and iodine) are low, which need to be improved for increasing the intake of these nutrients in public. For this, biofortification could make an impact, because it is sustainable and scalable approach to counter micronutrient malnutrition. In relatively remote rural areas where traditional foods are predominant in local diets and there is problem of food shortage during the off-season, the vegetable varieties with improved nutritional values and better storage-cum-transport life can serve much better. In this respect, breeding programmes for improving the content of nutrients and shelf life in vegetables are becoming more important for breeders. To improve such traits, it is desirable to (i) identify suitable donors in crop germplasm to develop genetic stocks for specific nutrients and nutraceuticals, (ii) identify genetics of the target compounds in donors and develop appropriate breeding strategy (iii) identify robust and tightly linked molecular markers for target traits as well as for background selection to track the introgression level and efficiency and (iv) deploy advance tools and techniques of biochemical analysis such as high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and Inductively coupled plasma mass spectrometry (ICP-MS) etc. for rapid detection of compounds in advance stage breeding materials.

Declaration

The authors declare no conflict of interest.

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