CURRENT APPROACHES FOR SMART AGRICULTURE

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AkiNik Publications

New Delhi

Published By: *AkiNik Publications*

AkiNik Publications
169, C-11, Sector - 3,
Rohini, Delhi-110085, India
Toll Free (India) – 18001234070

Editors: *Dr. Satybhan Singh, Dr. Himanshu Trivedi and Dr. Virendra Singh*

Edition: *1st*

Publication Year: *2022*

Pages: *516*

ISBN: *978-93-5570-290-6*

Book DOI: *https://doi.org/10.22271/ed.book.1833*

Price: *₹ 1765/-*

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Chapter - 33

Biotechnology: A Smart Way of Breeding Vegetable Crops

VK Dhangrah, YP Singh and Vimal Chaudhary

Introduction

Biotechnology refers generally to the application of a wide range of scientific techniques to the modification and improvement of plants, animals, and microorganisms that are of economic importance. The history of plant biotechnology could be traced back to the cell theory of Schleiden (1838) and Schwann (1839), and the discovery of genetic transformation in bacteria by Frederick Griffith, respectively. Early records of biotechnology process such as the fermentation and breeding practices which were inventions of the ancient Egyptians and other products like brew beer by the Sumerians; moldy soybean curds which was used as an antibiotic to treat boils by Chinese; practice of crop rotation to maximize soil fertility; use of powdered chrysanthemum an insecticide in China etc. However, the era of plant biotechnology is known to begin in the early 1980s with the mear stone reports of developing transgenic plants using *Agrobacterium tumefaciens* [Bevan *et al*. (1983); Fraley *et al*. (1983); Herrera-Estrella *et al*. (1983); Moose and Mumm (2008)]. The commercialization of transgenic crops revealed the successful integration of biotechnology into plant breeding and crop improvement programs by 1996 (Delannay *et al*., 1995).

Increasing world population and food demands require world agricultural production be increased by 50% by 2030. In the meantime, climate change and shrinking environmental resources are limiting agricultural production over the world. These challenges bring an urgent need to enhance crop productivity. To breed crops with increased yield and resistance to environment stresses, a pivotal consideration is how to effectively utilize genetic diversity. *Biotechnology* is one of the powerful and potential technology for bring desired changes in the characteristics of *plants, where there is a limited variation present.* The application of biotechnology to agriculturally important crop species has traditionally involved the use of selective breeding to bring about an exchange of genetic material between two parent plants to produce offspring having desired traits such as increased

yields, disease resistance, and enhanced productivity. Biotechnology provides farmers with tools that can make production cheaper and more manageable. For example, some biotechnology crops can be engineered to tolerate specific herbicides, which make weed control simpler and more efficient. Other crops have been engineered to be resistant to specific plant diseases and insect pests, which can make pest control more reliable and effective, and/or can decrease the use of synthetic pesticides. These crop production options can help countries keep pace with demands for food while reducing production costs.

It was estimated that 2.6 million deaths worldwide and 31 % of cardiovascular diseases may be attributed to inadequate consumption of fruit and vegetables according to WHO reports, 2002. Vegetables play an important role in human nutrition, being mostly low in fat and carbohydrates, but rich in vitamins, minerals, antioxidants and dietary fibres. By using conventional breeding several high yielding hybrid cultivars of different vegetable crops have been developed. It is gaining importance in breeding of vegetable plants to accomplish new varieties with high and stable yield, good quality, as well as pest and stress resistance. But, it requires many generations to get the desired combination of traits, and long-time lags restrict its further prospects. Such kind of selection based on the phenotype render a slow, demanding process and also expensive in terms of both time and money. Under such circumstances modern biotechnology proved an efficient tool in supplementing the conventional breeding methods of research and subsequently resulted in quantum jump in the improvement of productivity and nutritional quality of vegetable crops. Plant biotechnology not only accelerated the vegetable breeding programme but also extended the range of traits that can be addressed. By using biotechnological approaches, limitations of conventional breeding such as problem of linkage drag, sexual barrier in wide crosses, anti-nutritional factor etc. can be overcome efficiently and effectively. Recent developments in molecular biology such as in-vitro mutagenesis, genetic engineering, DNA sequencing, cloning, molecular marker etc. foster new meaning, new dimension, and new potential to old biotechnology. It also provided new perspectives of microbial intervention in agricultural practices, such as bio-fertilizers, bio-control agents, and various microbiological products used in modern agriculture (Tengerdy and Szakacs, 1998). Thus, modern biotechnology approaches can have a dramatic effect on the improvement of vegetable crops.

Methods of biotechnological applications in vegetable crops

The requirement of fruits and vegetables is increasing proportionally with the increasing population in the country. How do we keep horticultural

production on par with the burgeoning population? Although conventional plant breeding techniques have made considerable progress in the development of improved varieties, they have not been able to keep pace with the increasing demand for vegetables and fruits in the developing countries. Therefore, an immediate need is felt to integrate biotechnology to speed up the crop improvement programmes. Biotechnological tools have revolutionized the entire crop improvement programmes by providing new strains of plants, supply of planting material, more efficient and selective pesticides and improved fertilizers. Biotechnology has also been applied to improving the sensory properties and shelf life of vegetables. Many genetically modified fruits and vegetables are already in the market in developed countries. Some of the innovations, particularly in texture or flavor enhancement, have not been highly publicized as originating from plants modified by methods included in a broad definition of biotechnology. The major areas of biotechnology which can be adopted for improvement of vegetable crops include: Plant Tissue Culture, Genetic Engineering and Markers Assisted Breeding have been elaborated below.

Plant Tissue Culture: Plant tissue culture has a great significance in plant biotechnology especially in the crop improvement programmes. The term tissue culture may be defined as the process of in-vitro culture of explants (pieces of living differentiated tissues) in nutrient medium under aseptic conditions. Further, the discovery and understanding of role of plant growth hormones in the multiplication of cell also provided an extra aid for the development of in-vitro culture methods of plants. Different approaches of Plant Tissue Culture used for Vegetable Production have been illustrated in this context.

Micro-Propagation or *in - vitro* Propagation

Some of these enabling techniques are applied without resulting in a transgenic plant. These advanced technologies are sophisticated enhancements of methods that have long been common practice. For example, potatoes are propagated primarily by planting the buds, or "eyes," present on the tubers. This vegetative reproduction ensures that the new plants are identical to their parents. As one tuber can give only a few new plants, one for each "eye," several rounds of seed increase are needed to produce commercial quantities of potato "seed." Unfortunately, this also allows the transmission of disease-causing bacteria or viruses that may have infected the parent plant. Micro-propagation, the mass production of identical plants from tiny buds of the parent plant, is a bio-technique that can eliminate these pathogens from the progeny plants while retaining the advantages of vegetative reproduction.

Similarly, the individual cells of a plant can be separated, multiplied, and regenerated into whole plants through a process known as tissueculture. In this way, thousands of copies of a single plant can be made and certified pest-free. These techniques are valuable for propagating plants and are an essential component of more advanced methods of genetic modification. Several cultivars of garlic, potato, etc. have been known to be totally contaminated by viruses. In the absence of genetic resistance within the gene pool, meristem culture has been widely used to transform these cultivars into healthy cultivars, free from diseases. The principle is that a meristem (the apical part of a stem) is normally free from diseases. Its association with disease detection methods such as ELISA or PCR techniques makes it a very powerful tool to ensure propagation of healthy planting material. These include carrot, potato, celery, pepper, and melon varieties improved through applications of tissue culture.

Somatic Embryogenesis

The embryos formed from the somatic cells of plant in culture under *invitro* conditions are called as somatic embryos. Somatic embryogenesis under *in-vitro* conditions was first of all observed by Steward *et al.* (1958) in carrot (*Daucus carota*). Thereafter, somatic embryoids have been induced in many plants including vegetable crops like Garlic, Cucumber, Brinjal, Cauliflower, Coriander etc. The development of somatic embryo passes through the stages like globular, heart-shaped, torpedo-shaped and finally giving rise to the cotyledonary stage of somatic embryo.

Haploid Production

Using *in vitro* techniques, it is possible to regenerate plants from pollen or ovules. These plants, which contain only one copy of each chromosome, are called haploids. They are not viable. After appropriate chemical treatment, it is possible to restore the normal number of chromosomes and to regenerate viable plants. These plants, called double-haploids, are homozygous for all their genes. Such plants are of tremendous interest to plant breeders, since they allow development of pure line varieties or inbred parental lines much more quickly than through conventional breeding. Androgenesis (regeneration from pollen) has been successfully used for crops such as eggplant, pepper and wheat. Gynogenesis (regeneration from ovules) is used on barley. In addition, the *in-vitro* production of haploids also aids for: induction of genetic variabilities, disease resistance, salt tolerance, insect resistance, etc. Few examples are Capsicum, Beetroot, *Brassica pekinensis*, *B. chinensis*, etc.

Embryo Culture or Embryo rescue

Breeders need access to the largest possible genetic variability. In some cases, variability available within a given species is not sufficient to answer a specific problem (e.g. resistance to some new disease). A solution available to breeders is inter-specific hybridization (crossing plants from separate but related species). However, embryos resulting from such hybridization rarely survive, due to incompatibilities between the embryo and the mother plant. This technique has been used for the introduction of disease resistance into squash, lettuce, tomato, etc. It is used widely in the fields of agriculture, horticulture and forestry for production of hybrid plants. Embryo culture is advantageous for in-vitro micro propagation of plants, overcoming seed dormancy and for production of beneficial haploid plants.

Endosperm Culture (Triploid Production)

Endosperm tissue is triploid therefore the plantlets originating by the culture of endosperm are also triploid. In majority of flowering plant families (exceptions being *Orchidaceae, Podostemaceae, Trapaceae* which lack endosperm) the endosperm tissues are present. Endosperm culture has provided a novel strategy for plant breeding and horticulture for the production of triploid plantlets. It is an easy method for production of a large number of triploids in one step. The triploid plants are usually seedless therefore this technique is most beneficial for increasing the commercial value of fruits like apple, mango, grapes, watermelon, etc.

Somaclonal Variation

Somaclonal variations may be defined as those variations which occur in the cultured cells/tissues or plants regenerated from such cells *in-vitro*. These are usually heritable for qualitative as well as quantitative characters of plants. Somaclonal variants have proved as an alternate tool to plant breeding for production of improved varieties of plants. Gene mutations and changes in the structure, number of chromosomes are the main causes of production of somaclonal variants. It has been widely exploited for the improvement of asexually propagated vegetables as potato, tomato, onion, lettuce, etc. In potato, early blight resistant clones could only be identified by inoculating leaves of regenerated plants with toxin derived from *Alternaria solani.* In sexually propagated crops, chromosomal rearrangements sometimes cause infertility. Somaclonal variation is neither organ nor ex-plant specific in occurance, e.g. in potato Somaclonal variation has been observed in plants regenerated from leaf discs, rachis or petiole ex-plants. In tomato, Chopra and Narasimhulu (1990) reported that Somaclonal variation resulted in the

recovery of about 13 different nuclear mutations among the progeny of 230 regenerates. Since plant regeneration from somatic explants is relatively early compared to either gametic cells or protoplasts, somaclonal variation can play an important role in breeding of superior vegetable variety/hybrids.

Protoplast culture or Somatic Hybridisation

Somatic hybridization may be described as the production of hybrid cells by the fusion of protoplasts of somatic cells derived from two different plant species/varieties. Fusion of protoplasts is another technique to allow inter specific hybridization between species that cannot be crossed through conventional breeding, even using *in vitro* embryo rescue. Protoplasts are plant cells that have had their outer walls removed through chemical treatment. While it is difficult or impossible to fuse plant cells, it is possible through various techniques (using either chemical or physical treatments) to merge protoplasts from different crop species or genera, and then to regenerate a whole plant resulting from the fusion process. This technique has been used to introduce traits such as male sterility into rapeseed, or disease resistances in potato. It is immensely helpful for generating new and improved hybrid varieties of plant that may have characters of a completely different species. For example, 'Pomato' is a somatic hybrid which is produced by the fusion of protoplast of somatic cells from potato and tomato which are totally different species. Protoplasts were extracted and grown from the leaves of cauliflower cv. 7642B, which produces white curds even under direct sunshine.

Genetic Engineering

Developing plant varieties expressing good agronomic characteristics is the ultimate goal of plant breeders. With conventional plant breeding, however, there is little or no guarantee of obtaining any particular gene combination from the millions of crosses generated. In contrast, genetic engineering allows the direct transfer of one or just a few genes of interest, between either closely or distantly related organisms to obtain the desired agronomic trait. Genetic engineering offers plant breeders access to aninfinitely wide array of novel genes and traits, which can be inserted through a single event into high-yielding and locally-adapted cultivars. Genetically engineered plants are also being developed for a purpose known as phytoremediation in which the plants detoxify pollutants in the soil or absorb and accumulate polluting substances out of the soil so that the plants may be harvested and disposed of safely. For example, genetically engineered insect-resistant cotton has allowed for a significant reduction in the use of persistent, synthetic pesticides that may contaminate groundwater and the environment.

In terms of improved weed control, herbicide-tolerant soybeans, cotton, and corn enable the use of reduced-risk herbicides that break down more quickly in soil and are non-toxic to wildlife and humans. Herbicide-tolerant crops are particularly compatible with no-till or reduced tillage agriculture systems that help preserve topsoil from erosion. Moreover, there are wide spread concerns about the use of antibiotic and herbicide resistance genes as selectable markers from the point of view of ecological and human safety. Although transgenic tomato ‘‘Flavr Savr’’ was the first transgenic crop to be commercialized, but globally transgenics have been developed in case of many crops but the list dominantly includes Soybeans, Corn, Potatoes and vegetables like Tomatoes, Eggplant, Zucchini and Yellow Squash. Transgenic vegetable crops could make important contributions to sustainable vegetable production in this 21st century. However, the interaction of genetic makeup and environmental factors shapes the nature of all living things. When people eat a "healthy" diet, they are controlling environmental factors that will, within the limits of their genetic makeup, decrease their risk of developing a disease (Tietyen *et al*., 2000).

Marker-Assisted Breeding

Markers may be either phenotypic or genotypic, and marker-assisted breeding developed in the 1980s with the evolution of DNA marker technologies. Today, the main DNA markers used in breeding programmes are Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Microsatellites (SSRs), and Expressed Sequence Tags (ESTs). Each of these markers has a different set of advantages and limits. Cost and possible automation of the techniques are of particular importance for their adoption. Use of molecular markers, in association with linkage maps and genomics, offers plant breeders the potential to make genetic progress much more precisely and rapidly than through phenotypic selection. It also offers the possibility of addressing previously unattainable goals. There are many applications for the use of DNA markers in breeding programmes, which fall into four broad groups, based on the purpose of the intervention:

- enhancing knowledge of breeding material and systems, such as better understanding and more effective breeding of Quantitative Trait Loci (QTL);
- rapid introgression or backcross breeding of simple characters, as the number of back-crosses required can be reduced drastically if there are markers for the character to be introduced and for the genetic background of the recurrent parent;

- early or easy indirect character selection, which is important for genes that cannot be detected at an early development stage, such as high lysine and tryptophan genes in maize; and
- new goals not possible through traditional breeding, including pyramiding of disease resistance genes with indistinguishable phenotypes.

Marker-assisted breeding is also valuable for incorporating, or introgressing, specific desirable genes from wild relatives into domesticated varieties. Molecular markers are 'tags' that can be used to identify specific genes and locate them on the chromosomes. There are a number of different kinds of molecular markers that can be used to confirm the presence of a gene or even locate its relative position on the chromosome. Molecular markers for DNA allow the geneticist to "see" the genes and their arrangement on the chromosomes directly, without having to rely on the expression of the trait. An example of the use of biochemical markers in vegetable breeding is the introduction of the Root Knot Nematode resistance (*Mi*) gene into commercial tomato varieties. However, this section also contained a distinct form (or isozyme) of a common enzyme, acid phosphatase, that was directly adjacent (linked) to the *Mi* gene. In other cases, the gene of interest has been isolated, or cloned, and copies are available to use as the marker. An advantage of these types of markers is that they identify the DNA of the gene itself, rather than the expression of enzymes or proteins.

In addition, in many vegetables, including lettuce or tomato, relatively little variation is found among enzymes or proteins to use as markers to identify different lines, while a virtually inexhaustible number of DNA-based markers can be generated to distinguish among even closely related varieties. For example, there are at least 15 genes that can result in resistance to various strains of Lettuce Downy Mildew. In order to develop cultivars which possess several of these genes to give broad spectrum resistance, the rare individuals who inherit all of the desired genes from both parents must be identified.

Scope of biotechnology in vegetable breeding

Beginning in 1994, the first wave of products from biotechnological applications to vegetables was introduced in pilot test markets. Vine-ripe tomatoes with extended shelf life, processing tomatoes with superior quality and deep red color, squash with novel virus resistance, and potatoes genetically modified to produce an insect-killing protein are examples of the traits introduced into commercial vegetable varieties with the tools of biotechnology. Future impacts of biotechnology in crop production will be in the areas of:

Exploitation of male-sterility (MS)

In several plant species, genetic or cytoplasmic male sterility (GMS or CMS) leads to the suppression of production of viable pollen (Liedel and Anderson, 1993). MS has been observed in a wide variety of higher plants and is characterized by the very low level or the complete absence of pollen production. MS phenotype affects essentially the pollen producing organs because of the high-energy requirement of such tissues. The CGMS system has been commercially exploited in chilli, onion and carrot. In the recent past, chilli CGMS lines were introduced at the Indian Institute of Vegetable Research (IIVR) from AVRDC, which are utilized directly or indirectly to produce CMS-based hybrids, i.e Kashi Surkh (CCH-2) and Kashi Early (CCH-3). The Indian Institute of Horticultural Research (IIHR), Bangalore, India has also released chilli hybrids based on the CGMS system, i.e. Arka Meghna (MSH-172), MSH-149 and MSH-96. In carrot, the Indian Agricultural Research Institute (IARI) regional station, Katrain (HP), India has developed one hybrid, 'Pusa Nayanjyoti', which is based on petaloid CGMS. In the tropical group of carrot, IARI, New Delhi India has also reported CGMS system in different genetic back- grounds and evaluation of different hybrid combinations is in progress. In onion, IIHR, Bangalore has released two hybrids based on the CGMS system, i.e. Arka Kirtiman and Arka Lalima, and IARI, New Delhi has developed two hybrids in onion (Hybrid-63 and Hybrid-35). The CMS system has been commercially exploited in cabbage, cauliflower and onion (Hussain *et al*., 2018). A general characteristic of CMS is the dysfunction of mitochondria in tapetal cells. Mitochondrial genomes encoding chimeric proteins are presumably present in all tissues of the plant. Mitochondrial dysfunction produced by a chimeric protein interferes with the organelle function, and affects pollen production. Biotechnological approaches can be used to transfer CMS from within a species or from one species into another.

Resistance to insects, diseases and herbicides

Biotechnology will contribute to environmental quality protection by reducing the frequency of agricultural pesticide applications and by allowing more environmentally compatible materials and alternative methods to be employed. For example, plant-based pest resistance in both transgenic and conventionally selected varieties will reduce dependence on broad spectrum pesticides. The first transgenic plants with Bacillus thuringiensis (Bt) genes were produced in 1987 (Barton *et al*., 1987; Vaeck *et al*., 1987). Such genes include protease inhibitors, chitinases, secondary plant metabolites, and lectins (Hilder and Boulter, 1999; Sharma *et al*., 2000). Genes conferring

resistance to insects have been inserted into a wide array of crop plants including vegetables such as potato, tomato, brinjal, broccoli and lettuce (McLaren, 1998). Successful expression of Bt genes against the lepidopterous pests has been achieved in tomato, potato and brinjal. Serious concerns have also been raised about the safety of transgenic food itself. Most Bt toxins are specific to insects as they are activated in the alkaline medium of the insect gut. The Bt-proteins are rapidly degraded by the stomach juices of vertebrates. No major changes have been observed in the composition of the transgenic tomatoes and potatoes. Transgenic Bt tomatoes pose no additional risk to human and animal health. However, a number of aspects concerning the safety assessment of transgenic Bt tomatoes would require further study. The Fuchs *et al*. (2004) study examined the fitness costs of transgenic squash bearing CMV, ZYMV, and WMV potyvirus coat protein genes (Tricoli *et al*., 1995). An aphid-borne virus resistant to the transgenic plants was found to be common.

Tolerance to abiotic stresses

Development of crops with an inbuilt capacity to withstand abiotic stresses would help stabilize the crop production and significantly contribute to food security in developing countries. In bacteria, trehalose is produced by the action of trehalose phosphate synthase, which produces trehalose phosphate, and trehalose phosphate phosphatase-which degrades trehalose-6-phosphate into trehalose. When these two enzymes are expressed in transgenic plants, the plants have larger leaves, altered stem growth, and improved response to stress (Goddijn *et al*., 1998; Pilon-Smits *et al*., 1998). Over-expression of various glutamate dehydrogenases (GDH) also improves plant growth and stress tolerance. Plants have been specifically transformed with genes encoding the a-and b-subunits of the chloroplast-located GDH from the alga, *Chlorella sorokiniana* (Schmidt and Miller, 1997). Plants with an ability to produce more citric acid in roots provide tolerance to aluminium in acid soils (De la Fuente *et al*., 1997). Introduction of functional calcineurin activity provides tolerance to salinity (Pardo *et al*., 1999) involving the introduction of a gene encoding a plant farnesyltransferase (Pei *et al*., 1998) and inhibitors of this enzyme when expressed in plants, enhance drought tolerance, delay senescence, and modify the growth habit. A salt tolerance gene isolated from mangroove (*Avicennia marina*) has been cloned, and can be transferred into other crop plants (Swaminathan, 2000). The gutD gene from *Escherichia coli* can also be used to provide salt tolerance (Liu *et al*., 1999). These genes hold a great potential for increasing crop production in marginal lands.

Nutritional factors and post-harvest quality

Several quality traits can be targeted to improve the nutritional status of crop produce. These include carbohydrates, proteins, oils, vitamins, iron, and amino acids. The selection of target traits is influenced by the end users, producers, and agro-based industry. Altering protein levels, composition of fatty acids, vitamins and amino acids is being increasingly targeted for value addition in vegetable crops like potato. Decreasing the amounts of oligosaccharides (such as raffinose and stachyose) improves digestibility, and decreases the degree of flatulence during digestion. Transgenic technology can also be used to remove anti-nutritional factors (Kaufman *et al*., 1998). Flavr Savr, the variety of tomato developed with increased shelf life through genetic modification to regulate the expression of the enzyme polygalacturonase (PG) in ripening tomato fruit. This enzyme is one of the most abundant proteins in ripe tomato fruit and has long been thought to be responsible for softening in ripe tomatoes. In watermelon, using CAPS, which is a form of cleaved amplified polymorphic sequence (CAPS), Bang *et al*.(2007) identified a key gene, lycopene beta cyclase (LCYB), that determines the color of canary yellow and red watermelon flesh. The gene for the extremely sweet protein "thaumatin II" from the plant species of Africa, *Thaumatococcus daniellii* was utilized to increase carrot flavour.

Understanding nature of gene action and metabolic pathways

The last decade has seen that systematic whole genome sequencing will provide critical information on gene and genome organization and function, which will revolutionize our understanding of crop production and the ability to manipulate those traits contributing to high crop productivity (Pereira, 2000). Advances in these areas will fuel the mapping of QTL (quantitative trait loci) underlying agronomic traits in less studied crops. The use of QTL markers in crop improvement promises rapidly and efficient utilization of novel traits from closely related wild species. It takes five to six generations to transfer a trait within a species into the high yielding locally adapted cultivars through the conventional breeding, and one has to plant a large number of progenies to be able to select the plants with appropriate combination of traits. The improved lines developed then have to go through a set of multi-location tests, before a variety could be identified for cultivation by the farmers. This process takes minimum of 7_/10 years. However, genetic transformation provides access to genes from other species, which can be used for producing transgenic crops, ability to change the level of gene expression, and capability to change the spatial and temporal pattern of gene expression. The genes of interest can be transferred into the target crops/cultivars in a

single event, and it takes 5_/6 years to develop cultivars with stable gene expression. The lines thus produced can be released for cultivation by the farmers or used as donor parents in the conventional plant breeding and/or marker assisted selection. In the marker-assisted selection, the elite lines can be crossed with another line having trait(s) of interest. The marker assisted selection takes 3_/6 years, and thus speeding up the pace of transferring the traits of interest into the improved varieties, and it does not require large scale planting of the progenies up to crop harvest, as the plants showing the presence of the trait or QTL only need to be maintained up to maturity. Quantitative trait loci (QTLs) for diverse qualitative and sensory characteristics, including as taste, fragrance, and texture, were mapped using molecular markers in tomato (Pradhan *et al*., 2021).

In understanding of metabolic pathways, sucrose phosphate synthase (SPS) is a key enzyme for the regulation of sucrose metabolism. Modification of the activity of metabolites of the TCA (tricarboxylic acid) cycle by reducing the amount of the NAD-malic enzyme can also be used for increasing starch concentrations (Leaver *et al*., 1998). Introduction of the Escherichia coli inorganic pyrophosphatase to alter the amount of sugar (Sonnewald and Willimitzer, 1996), and modification of hexokinases (Sheen and Jang, 1997), which affect the sugar-sensing capacities of a plant as well as sucrose binding proteins (Grimes and Chao, 1998), and a class of cupin protein (Dunwell, 2000) have been implicated in sugar unloading in developing legume seeds. Both plant and non-plant genes have been introduced into test tomato varieties to increase sucrose accumulation, increase the conversion of sucrose to fructose in the fruit, and sustain organic acids during ripening of tomatoes. This has opened up exciting possibilities for changing the chemical composition of especially root and tuber vegetables to meet specific requirements.

DNA marker-assisted selection

Recombinant DNA technologies, besides generating information on gene sequences and function, allows the identification of specific chromosomal regions carrying genes contributing to traits of economic interest (Karp *et al*., 1997). The identification of DNA markers for traits of interest usually depends on making crosses between two genotypes with substantial and heritable differences in trait(s) of interest. This approach can only be used when parental genotypes can be identified with opposing phenotypes for the trait of interest. Interspecific crosses can be used to good effect in this respect, but linkage maps derived from such crosses may have limited relevance in crop breeding programs (Fulton *et al*., 1997). Once genomic regions contributing to the trait

of interest have been assigned and the alleles at each locus designated, they can be transferred into locally adapted high-yielding cultivars by making requisite crosses. The offspring with a desired combination of alleles can then be selected for further evaluation using marker-assisted selection. Wild relatives of commercial crops contain alleles of importance for improving crop performance and resistance to biotic and abiotic stress factors, and these can be effectively incorporated into crop breeding programs through marker-assisted selection (Xiao *et al*., 1996). DNA marker technology has been used in commercial plant breeding programs since the early 1990s, and has proved useful for the rapid and efficient transfer of these traits into agronomically desirable varieties and hybrids (Miflin, 2000). The use of DNA markers for indirect selection offers greatest potential gains for quantitative traits with low heritability as these are the most difficult characters to work with in the field through phenotypic selection.

Altering senescence

Leaf senescence leads to a progressive death of the leaf or a plant upon aging due to reduction in the production in cytokinin, Cytokinin is a plant hormone that naturally prevents senescence and maintains photosynthetic activity in leaves. Reduction in leaf senescence (Smart *et al*., 1996; De Nijs *et al*., 1997) would improve the performance of a plant, and thereby increase the crop yield. This in part can be achieved through stay green leaves in leafy vegetables like palak, amaranth, etc. Stay green trait in cluster bean is also associated with adaptation to drought stress. Introduction of farnesyl transferase and isopentenyl transferase (IPT) genes delays senescence (Amasino and Gan, 1997). The process of leaf senescence can be blocked through a gene encoding the cytokinin-synthesis enzyme, isopentenyl transferase. When transformed with the promoter, SAG12-IPT, a plant will produce enough cytokinin to delay leaf senescence. Commercial uses for delayed senescence include increasing plant vegetative growth, seed and fruit production, prolonging the shelf-life of vegetables, provide a safe and natural source of cytokinin.

Increased Photosynthetic efficiency and improved yield

An exciting experimental approach to increase crop yield radically is to change components of plant biochemistry with respect to introducing the C4 type of photosynthesis into a C3 plants such as potato (Ishimaru *et al*., 1998). C3 photosynthesis suffers from O2 inhibition due to the oxygenase reaction of ribulose 1, 5-biophosphate carboxylase/oxygenase (Rubisco), and the subsequent loss of CO2 from photorespiration. In contrast, C4 plants such as

amaranth have evolved a biochemical mechanism to overcome this inhibition. A key feature of this mechanism is the activity of phosphoenolpyruvate carboxylase (PEPC), an enzyme that fixes atmospheric CO2 in the cytosol of mesophyll cells (Grula and Hudspeth, 1999). Using an Agrobacterium-mediated transformation system, the intact C_4 plants PEPC has recently been transferred into the C3 plants (Matsuoka *et al*., 1998; Miyao and Matsuoka, 1999). Appropriate manipulation of the enzymes involved in photosynthetic activity can be used to increase the productivity potential of C3 plants. Manipulation of chlorophyll a/b binding genes has also been used to modify chlorophyll amounts (Grimm, 1998; Johnson-Flanagan *et al*., 1998).

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