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Research Article

Role of Biotechnology in Vegetable Breeding

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Abstract

Biotechnology generates efficient and cost-effective revenue for the production of a variety of unique, value-added products and tools by utilizing living organisms to manufacture or change items, to improve plants and animals, or to develop organisms for specialized applications. In the last two decades, it has created unrivaled opportunities and has risen to become the world's fastest and most rapidly rising technology in general and in agriculture in particular. It has the potential to increase food productivity while simultaneously decreasing agriculture's reliance on chemicals, lowering the cost of raw materials, and reducing the negative environmental impacts associated with traditional production procedures. Abiotic and biotic challenges are addressed by virtue of recombinant DNA (r-DNA) technology. Similarly, quantitative trait loci (QTL) mapping and tissue culture techniques are helping to improve crop quality at the molecular level.The current understanding of new tools for deciphering encoded genetic languages underpins the potential applications of this technology in vegetable crop breeding.

***Key words**:* Quantitative trait loci (QTL), Recombinant DNA (r-DNA) technology, Tissue culture.

Introduction

Biotechnology has recently created unique prospects in the field of science not only for the manipulation of biological systems for the use of human beings, several studies has been undertaken for the betterment of human beings, but subsequently it has become the fastest and quickly growing technology in the world.

This technology is capable of providing skilled and affordable methods to make an assortment of new, valuable agricultural products and implements. It is possible to boost food output, reduce the use of chemicals in agriculture, reduce the costs of raw materials, and minimize the environmental impacts that are connected with traditional approaches to product development through the employment of living organisms (US National Science Foundation).

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In addition, now biotechnology can make foods healthier and more nutritious, as it is used to improve the quality of seed grains, increase protein levels and resistance to pathogens, insect-pests as well as tolerance to abiotic stresses like droughts, floods and extreme temperatures, *i.e.* tomato has become a popular plant system because of the advanced state of its classical and molecular genetic characterization.

Biotechnology is a science which depend on advances in Biology, Chemistry, Geneticsand Genetic Engineering for its successful application in the field of agriculture.Combination of Molecular markers, genome mapping, Recombinant DNA technologies, Cell and tissue culture for improvement of vegetables that forms the basis of genetic engineering of microbes, plants and animals. For the improvement of vegetable crops, tissue culture is a potential source of inducing genetic variation such as gametoclonal, somaclonal and proto clonal observed among plants.

In vitro culture

The tomato belongs in the nightshade family, which includes key commercial plants such as potatoes, eggplants, tobacco, petunias, and peppers (Bai and Lindhout, 2007). For quite some time, scientists could not decide on whether the tomato was a fruit or a vegetable. Genetic data (genome mapping) and morphological information were utilized to verify the Solanaceae classifications, with the Lycopersicon genus (Lycopersicon section) being used as the proof (Foolad, 2007).

Because of the length of time required for breeding, traditional approaches of improvement are difficult and time-consuming. On top of that, good breeding criteria are tough to pick. It is thus a must for using cell and tissue culture for genetic improvement to use simple and effective regenerative methods (genetically transformed plants for commercial applications).High-value commercial cultivar cloning, virus-free plants, and genetic alteration have all been used successfully using tomato in vitro cultivation (Li *et al.* 2011; Yarra *et al*. 2012; Namitha and Negi, 2013). Genetically modified tomato (FlavrSavr) was the first commercially manufactured food that received human consumption clearance. In 1994, the FDA approved the FlavrSavr tomato. The tomatoes tasted unremarkable and were unusually delicate, making shipping challenging. The first GM plant to be licensed for marketing in China was the GM tomato Huzahong No. 1 (from Huzahong Agricultural University), which has increased shelf life properties.Other tomato varieties that have been approved in certain countries (the United States, Japan, Mexico, and Canada) include Agritope Inc's 351N, Monsanto's 8,338 and 5,345, DNA Plant Technology Corp's 1345-4, and Zeneca Seeds' B, Da, and F. Table 1 provides a comprehensive description.

Table 1. Transgenic tomato varieties approved for commercialization. Based on Yang *et al.* (2005) and Fukkuda-Parr, (2012)

Company	Event	Trait	Year	Approved for	Country
Calgene	FlavrSavr CGN-89564	Delayed softening (developed by additional PG gene expressed)	1994	All uses in USA; Japan and Mexico for feed and for	USA

				environment	
Calgene	FlavrSavr N 73 1436-11	Delayed ripening (developed by additional PG gene expressed)	1996	All uses in USA	USA
Monsanto	8338	Delayed ripening (developed by introduction of 1-aminocyclopropane-1-carboxylic acid deaminase (accd) gene)	1995	All uses in USA	USA
Beijing University	PK-TM8805R (8805R)	Delayed ripening	1999	Food, feed, cultivation in China	China

Studies have found that high quantities of vitamins C and E and βcarotene in vegetables, such as tomatoes, may help protect against illness risk, including malignancies and heart disease (Singh *et al.,* 1998).It involves a multi-disciplinary approach to science. Biotechnology arrived on the scene in the late 1970 and early 1980 when the several major advances in science suddenly demonstrated enormous potential for its application.

In potato, it is possible to develop virus free plants by meristem tip culture. As under field conditions screening of germplasm is time consuming and expensive, barriers in sexual hybridization and therefore many inter specific and inter generic hybridizations are not successful , but it is economical and convenient at the cellular level. Such hybridizations have now been made successful in a few cases by protoplasts fusion. Similarly, maintenance of germplasm through traditional methods is expensive and by application of tissue culture, germplasm can be maintained and stored economically. Furthermore, it offers a scope to generate geneic variability through tissue culture techniques (Murashige, 1974 and Sinks andReynolds, 1986).

Somaclonal variation

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.

Protoclonal variation

As compared to callus culture the range of variation among protoclones is significantly higher. Shepard and associates (1980)screened variability in over 1000 protoclones of potato variety Russett Burbank for the traits growth habit, maturation period, tuber uniformity, skin colour, etc. Some of the protoclones were resistant to symptoms caused by *Alternaria solani* toxin. Lines resistant to late blight were also recovered. Evaluation of 65 selected protoclones for 35 characters indicated significant variation for 22 characters.

Some useful plant variations obtained through somaclonal variation in vegetables (Kalloo, 1988)

Plant	Improvement
Carrots	Improved snacking characteristics (sweetness, crunchiness, crispness).
Lettuce	Leaf shape and leaf colour improved snacking characteristics.
Celery	Improved snacking characteristics.
Potato	Resistance to early and late blight, tuber shape, growth habit and tuber colour.
Sweet potato	Variety ‘Scarlet’ developed, which is resistant to *Fusarium oxysporum* f. *Batatis* and give higher tuber yields.
Tomato	Increased solids content, jointless pedicel and male sterility.
Onion	Bulb shape, size and plant height.

Resynthesis of *Brassica napus* is accomplished by fusing protoplasts of B. oleracea and B. campestris (Pelletier *et al.*, 1983). Additionally, a similar result can be obtained by adding cytoplasmic male sterility genes to the vegetable onion and carrots to make hybrid seeds affordably.

Kuchke *et al.* (1983) and Helgeson *et al.* (1986) developed hexaploid hybrids resulting from protoplast fusion of *Solanum brevidem . S. tuberosum*, which showed resistance to leaf roll virus and race 'O' of late blight.

Gleddlie*et al*. (1986) could fuse protoplasts of *Solanun melongena* and *S. sismbrifollum* (resistant to phomopsis blight). They observed that this resulted in aneuploids and hybrid was sterile. Hybrid plants are formed by hybrids between members of the same family that are genetically incompatible. These hybrids have the same number of chromosomes as their parents. There are many popular ones, but the one that is most well-known is the "pomato" which is a tomato-potato somatic hybrid.

Tissue culture techniques in vegetables

Plant organ used	Vegetables
Callaus/tissue culture	Lettuce, sweet potato and cucumber
Cell culture	Garlic
Meristem culture	Onion and cauliflower
Organ culture	Lettuce, tomato, brinjal, sweet potato and cucumber

Embryo culture	Tomato, okra, French bean, Brassicas and pumpkin
Anther and pollen culture	Potato, tomato, peas, asparagus, French bean, Brassicas and capsicum etc.

Cell and tissue culture to potato improvement

Tissue culture is the *in vitro* regeneration of plants from disease-free plant components (cells, tissue). This method enables the generation of disease-free agricultural planting material. Cell and tissue culture methods have been used more frequently in potato during the last 50 years than in any other crop species. In vitro cultures of potato have been produced from various plant components such as petioles, ovaries, anthers, stems, roots, and shoot tips since 1951 (Steward and Caplin, 1951), as mentioned by Srivastava *et al.,* 2012; Kumar *et al.,* (2015); (Bajaj, 1987). A variety of approaches had been refined in this crop throughout the years because to its great amenability to in vitro modifications. These approaches come in a variety of levels of sophistication, constituting a whole range of technologies. While some of these technologies, such as micropropagation and pathogen eradication, have been used to increase potato output, others are currently being developed and improved. The most common use in potato is the employment of in vitro techniques for virus eradication (meristem culture) and clonal mass multiplication (micropropagation). In many places, disease-free potato clones generated in vitro coupled with traditional multiplication methods have become an important element of seed production (Naik and Sarkar, 2000).

Potato conventional breeding	**Genetically engineered potato**
Gene pool restricted by sexual compatibility	Gene pool not restricted, any organism can be used as gene donor
Introduction of additional, undesirable genes	Introduction of the desired gene only
Gene of interest is usually not known and cannot be modified	Gene of interest is well characterized and can be improved
Unknown recombination events	Well-characterized recombination events
10-15 years needed	Between 3 and 5 years for the development of a transgenic potato variety
Breeders' rights allow seed multiplication for own use and breeding	Patents rights are highly restrictive for users
Thought io be safe because of familiarity	Fear of novelty and unknown
Accessible to almost everyone	Still in the hands of developers
Developed in many countries	Still mostly developed in Northern countries

Utilization of biotechnological tools in breeding for quality traits

Tomato:

Quantitative trait loci (QTLs) for diverse qualitative and sensory characteristics, including as taste, fragrance, and texture, were mapped using molecular markers. The researchers used introgression line analysis to identify the positions of B on the standard tomato linkage map, correlating them with two CAPS and SCAR markers. In a marker-assisted breeding program, these markers might be used to trace the B gene introgression from orange-fruited L. *cheesmanii* accession LA317 into red-fruited cultivated tomato breeding lines and generate new tomato genotypes with higher beta carotene content and greater nutritional value.

Found QTLs impacting the variance of tomato quality attributes by using cross-pollinated tomato of cherry tomatoes that had a sweet flavor, but small size, and a low-quality line. The weight, color, and hardness of fruits were measured as well as other chemical measurements of fruit dry matter, acidity, pH, TSS, sugar, lycopene, carotene, and 12 aroma volatiles. Descriptive sensory profiling was also used to assess the lines (taste, texture and aroma). For all of the characteristics, a number of QTLs were discovered, some of which had significant impacts. In general, the bulk of the positive alleles found in the cherry tomato parents were for chemical and sensory properties, which points to this line as a good candidate for boosting tomato organoleptic quality.Previously, the concentrations of soluble solids in 100 F3 families of a cross between the L. esculentum cv. UC82 and the wild *L. chmielewskii* accession LA1028 were examined. They discovered seven RFLP markers linked to soluble solids expression. For soluble solids concentration, indirect selection based on RFLP genotypes successfully discriminates across F3 families.

There are two sets of basic traits present in tomato fruits: both their organoleptic aspects (taste, smell, etc.) and their nutritional value. All aspects of the fruit's texture, taste, and aroma are organoleptic. Tomato fruits, as an excellent source of a wide range of vitamins and minerals, as well as sugars, flavonoids, ascorbic acids, folate, and carotenoids, are a great nutritional choice, providing around five grams of dietary fiber, one gram of fat, one hundred calories, and virtually no sodium. Genetically modified tomatoes demonstrated the effect of expansin or b-galactosidase on fruit softness and firmness according to the findings of Brummell *et al.* (1999) and Smith *et al.* (2002).

Scientists have increasingly focused their attention on the nutritional characteristics of tomato fruits, in addition to their organoleptic qualities. Zanor *et al*. (2009) used an RNAi method in transgenic tomato plants to show that cell wall invertase (LIN5) is involved in regulating the amount of soluble solids.

Brinjal:

By changing auxin levels in developing ovules of the transgenic egg plant utilizing Agrobacterium tumefasciens transformation, fruit quality enhancement in brinjal may be achieved by genetic manipulation for parthenocarpy.

Because eggplant can be easily altered in vitro, it has been effectively genetically modified using an Agrobacterium tumefaciens vector since the 1980s. So far, genetic changes have primarily focused on insect resistance and abiotic stress tolerance.

An artificial scream At the NRC for Plant Biotechnology, IARI, New Delhi, 1 Ab gene coding for an insecticidal crystal protein (ICP) of Bacillus thuriengenesis (Bt) has been transferred to brinjal. Gene integration and m-RNA expression were established in hybridization studies. Bt toxin protein expression in transgenic plants was examined using a double antibody sandwich ELISA. The transgenic brinjal fruits have a considerable insecticidal effect against fruit borer larvae as a result of the expression. The findings also showed that a synthetic gene for insect control based on monocot codon use may be produced in dicotyledon plants (Kumar*et al*. 1996). The transgenic material exhibited substantial insecticidal action against the insect larva in Asia and was produced with a synthetic cry 1Ab gene coding for an insecticidal crystal protein of Bt.

Using a Bt gene expressing for the cryIIIB toxin, resistance to the colarado beetle (*Leptinotarsa decemlineata*), one of the few plant pests against which no eggplant germplasm resistance has been identified, resistance to the colarado beetle (*Leptinotarsa decemlineata*) has been successfully developed (Tripathy *et al.*, 2020; Tripathy *et al.*, 2020).

Hot and Sweet pepper

Initially, Kim *et al*. (2001) used a suppression subtractive hybridisation technique to isolate 39 cDNAs possibly related to pungency, and SB2-66 showed placental specific expression and similarities to some acyltransferases enzymes. Later, when cDNA AT3 was characterized, the SB2-66 fragment was found within the sequence. The AT3 gene encodes the Pun1 gene, which is a recessive gene that controls the presence or absence of pungency as a switch on/off paradigm. In non pungent genotypes, a 2.5 kb loss spanning the promoter and first exon results in the lack of pungency. Catf 1 and Catf 2 are two more potential Capsicumacyl-transferase genes that have been linked to pungency. Prasad *et al.* (2006) identified and characterized csyl 1, a key gene involved in the condensation of vanillylamine with a branched chain fatty acid in the biosynthesis of capsaicinoids. A single nucleotide polymorphism (SNP) was linked to the pungent trait and was found to be effective in identifying pungent genotypes.

Melons

A melon is one of the most popular fruits. The breakdown of chlorophyll, disintegration of the cell wall, rise in sugar content, alteration in pigment production, and accumulation of flavor and aromatic chemicals are only few of the complicated developmental processes involved in fruit development and ripening. 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, and Bang *et al.* (2007) and CAPS are highly suitable for identifying canary yellow and red watermelon fruit at the seedling stage.

Carrot

The gene for the extremely sweet protein "thaumatin II" from Thaumatococcusdaniellii was utilized to increase carrot flavor. Using disarmed highly virulent Agrobacterium strain CBE21, a binary vector for expression in carrot was created based on the coding sequence. PCR analysis verified the insertion of the thaumatin gene. Carrot roots had a high level of Thaumatin II expression.

Cauliflower

Protoplasts were extracted and grown from the leaves of cauliflower cv. 7642B, which produces white curds even under direct sunshine. The majority of protoplast derived lines produced curd sooner than their seed plant counterparts, and several of the early protoplast derived lines produced curd with acceptable size, color, shape, and hardness.

Chinese cabbage

For breeding to increase the nutritional quality of Chinese cabbage, genetic dissection of leaf mineral accumulation and resistance to zinc stress is critical. The genetics of mineral buildup and the growth response to zinc were studied using a mapping population of 183 double haploid (DH) lines. 203 AFLPs, 58 SSRs, 22 SAPs, and 4 ESTPs were used to create the genomic map. In the leaves of 142 DH lines growing in an open field, the content of 11 minerals was measured. In addition, shoot dry biomass (SDB) was studied in a hydroponics experiment under normal, inadequate, and excessive zinc nutritional circumstances. Multiple QTL model (MQM) mapping found ten QTLs that explained 11.1-17.1% of the variation in Na, Mg, P, Al, Fe, Mn, and Zn concentrations. Only under normal, deficient, and severe stress was one common QTL discovered to impact shoot dry mass (SDB).

Garlic

The expression of esterase isozymes in cloves from winter and summer ecotypes of boring and non-bolting garlic clones was studied. Vertical block electrophoresis on polyacrylamide gels was used to acquire the isozymes patterns. There were quantitative and qualitative variations between loci of winter and summer garlic ecotypes, indicating that isoesterases can be used to identify garlic clones.

Pumpkin/Squash

Pumpkin, also known as squashes, is a common vegetable grown all over the world that originated in South America and has the benefit of being able to be preserved for a long time at room temperature. It's chock-full of antioxidants, beta-carotene fiber, minerals, and vitamin C. As a result, cucurbits must be transformed utilizing biotechnology to create resistance and great potential.

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). Aphid-borne viruses resistant to the transgenic plants was found to be common. To show that transgenes, which lead to gene flow and hybridization, have a selective value, the authors state that CMV, ZYMV, and WMV can seriously hamper the development and replication of wild squash populations.

Cucurbita pepo has a B gene that causes premature yellow fruit coloring before anthesis, and this gene has been effectively transferred to *Cucurbita moschata*. A squash genetic map was created, and the associated marker of the B gene was 110 - 1700, with a recombination distance of 27.1 cM (centi Morgan).

Both Zucchini Yellow Mosaic Virus and Watermelon Mosaic Virus, diminish the yield and fruit quality, resulting in crops that are unmarketable (Clough and Hamm, 1995). Fuchs and Gonsalves performed field tests of transgenic yellow crookneck squash lines expressing ZYMV and WMV2 CP genes to find out whether or not they are resistant to these two viruses (Fuchs and Gonsalves,1995). A higher disease pressure was provided by using mechanical inoculations and aphid vector challenge inoculations.

Conclusion

Aside from making major contributions to the evolution of vegetables, biotechnology approaches have also been shown to be insufficient to completely replace conventional breeding methods. In the event that normal breeding processes do not produce the intended results, several strategies are employed. They are not more generally applied because of two primary seasons: first, most vegetables do not allow for the regeneration of large numbers of plants and second, because the processes require a high degree of expertise and well-equipped facilities, they are still in the early stages of development, with a slew of challenges to be addressed. The great genetic variability found in both wild and cultivated vegetable species makes it possible to introduce novel genes into the plant genome through biotechnology. Melons such as cantaloupes and muskmelons, which are highly perishable after ripening, have a lot of room to improve their quality characteristics, which include antioxidant components such as β-carotene and carotenoids such as lycopene and anthocyanins, as well as the shelf life of melons such as watermelon, muskmelon, and cucumber. A key characteristic could be the consumer's choice for the size, shape, and appearance of the fruit, on the other hand.

References

1. Bai, Y., Lindhout, P. (2007). Domestication and breeding of tomatoes: what have we gained and what can we gain in the future. Ann Bot 100:1085–1094.
2. Bajaj, Y.P.S. (1987). Biotechnology and 21st century potato. In: Biotechnology in Agriculture and Forestry. Vol. 3: Potato (ed. Bajaj, Y. P. S.), Springer-Verlag, Berlin. pp. 3-22.
3. Bang H, Kim S, D Leskovar and King S . (2007). Development of a codominant CAPS marker for allelie selection between canary yellow and red watermelon based on SNP in lycopene β-cyclase (LCYB) gene. Mol.Breed.2063-72.
4. Brummell, A.A., Harpster, M.H., Civello, P.C., Palys, J.M., Bennett, A.B., Dunsmuira, P. (1999). Modification of expansion protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11:2203–2216.
5. Chopra,V.L. and Narsimaulu, S.B.(1990). Biotechnology for crop improvement. In Chopra V.L. and Nasim Anwar Eds. Genetic engineering and Biotechnology concepts, methods and application, Oxford Publication, IBH Publishing, New Delhi, 159-180 pp.
6. Clough, G.H. and Hamm, P.B. (1995). Coat protein transgenic resistance to watermelon mosaic and Zucchini yellow mosaic virus in squash and cantaloupe. Plant Disease.,79:1107-1109.
7. Foolad, M.R.(2007). Genome mapping and molecular breeding of tomato. Int J Plant Genomics. 64358:52
8. Fuchs, M. and Gonsalves, D.(1995). Resistance of transgenic hybrid squash ZW-20 expressing the coat protein genes of Zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses. Biotechnol. 13:1466-1473.
9. Fuchs, M., Chirco, E.M., Jim, R., Mcferson, J.R. and Dennis Gonslves, D.(2004). Comparative fitness of a wild squash species and three generations of hybrids between wild × virus-resistant transgenic squash. Environ Biosafety Res. 3:17-28.
10. Fukkuda-Parr S .(2012). The green revolution: GM crops and unequal development. UK Bath Press, Bath
11. Gleddie, S., Keller, K.W., Setterfield, G.(1986). Production and characterisation of somatic hybrids between *Solanum melongena* L. and *S. sisymbrifolium* Lam., Theor. Appl. Genet. 71: 613/ 621.
12. Hanus-Fajerska, E. (2006). Variation in tomato plants regenerated from Cucumber Mosaic Viruse infected tissue. ISHS Acta Hort789: XV Meeting of the EUCARPIA Tomato Working Group. http:// www.actahort.org/books/789/789_40.htm
13. Helgeson, J. P., Hunt, G. J., Haberlachm G. T. and Austin, S. (1986). Somatic hybrids between *Solanum brevidens* and *Solanum tuberosum*: Expression of a late blight resistance gene and potato leaf roll resistance. Plant Cell Rep. 3: 212–214.
14. Kalloo, G. (1988). Vegetable Breeding. VoL I. CRC Press Inc. Boca Raton, Florida. Ppl05-135.
15. Kim, M., Kim, S., Ki, B.D. (2001). Isolation of cDNA Clones Differentially Accumulated in the Placenta of Pungent Pepper by Suppression Subtiractive Hybridization. Molecules and Cells. 11(2):213-9

16. Kumar, P.A., Mohapatra, T., Archak, S., Sharma, R.P.(1996). Expression of *Bacillus thuringiensis* δ-endotoxin in transgenic tobacco and cauliflower. In: Chopra VL, Sharma RP, Swaminathan MS (eds) Agricultural Biotechnology, pp. 191–197, Oxford-IBH, New Delhi
17. Kumar, V., Rout, S., Tak, M.K. and Deepak,K.R.(2015).Application of Biotechnology in Forestry: Current status and future perspective. Nature Environment and Pollution Technology.14 (3):645-653.
18. Li, T., Sun, J.K., Lu, Z.H., Liu, Q. (2011). Transformation of HBsAg (Hepatitis B Surface Antigen) gene into tomato mediated by *Agrobacterium tumefaciens*. Czech J Genet Plant Breed 2:69–77
19. Murashige, T. (1974) Plant Propagation through Tissue Cultures. Annual Review of Plant Physiology. 25: 135-166.
20. Naik, P.S. and Sarkar, D. (2000). *In vitro* propagation and conservation of genetic resources in potato. In: Biotechnology in Horticultural and Plantation Crops, (eds. Chadha, K.L., Ravindran, P.N. and Sahijram, Leela) Malhotra Publishing House, New Delhi, pp. 369-406.
21. Namitha, K.K., Negi, P. (2013) Morphogenetic potential of tomato (*Lycopersicon esculentum*) cv. ArkaAhuti to plant growth regulators. Notulae Scientia Biologicae 2:220–225
22. Pelletier, G., C. Primard, F. Vedel, P. Chetrit, R. Remy, P. Rousselle, and M. Renard. (1983). Intergeneric cytoplasmic hybridization in Cruciferae by protoplast fusion. Mol. Gen. Genet. 191:244–250.
23. Prasad, B. C. N., Gururaj, H. B., Kumar, V., Giridhar, P., Parimalan, R., Sharma, A., Ravishankar, G. A.(2006). Influence of 8-methyl-nonenoic acid on capsaicin biosynthesis in *in-vivo* and *in-vitro* cell cultures of Capsicum spp. J Agric Food Chem. 8:54(5):1854-9.
24. Shepard, J.F., Bidney, D. and Shahin, E.(1980). Patato proroplast in crop improvemet. Science.208:17-24.
25. Singh, C., Vishwanatham, M. K., Nirmal, Kumar ., Agarwal, M. C. (1998). Growth, survival and mean annual increment of *Quercus leucotrichophora* (banj oak) on degraded lands of Doon Valley. Indian Forester. 124 (9): 732-738.
26. Sink, K.C., Reynolds, J.F. (1986). Tomato (*Lycopersicon esculentum*). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry 2. Crops I. Springer, Berlin Heidelberg New York. pp 319–344
27. Smith, D.L., Abbott, A.A., Gross, K.C. (2002) Down-regulation of tomato b-galactosidase 4 results in decreased fruit softening. Plant Physiol 4:1755–1762.
28. Srivastava, A.K., Diengdoh, L.C., Rai, R., and Bag, T.K. (2012). Tissue Culture – Technology Harnessed for Potato Seed Production. Central Potato Research Station, 5th Mile, Upper Shillong, Shillong.793 009.
29. Steward, F.C. and Caplin, S.M.(1951). Tissue culture from potato tuber: the synergistic action of 2,4-D and of coconut milk. Science. 111:518-520.
30. Tricoli, D.M., Carney, K.J., Russell, P.F., McMaster, J.R., Groff, D.W., Hadden, K.C., Himmel, P.T., Hubbard, J.P., Boeshore, M.L. and Quemada, H.D.(1995). Field evaluation of transgenic squash containing single and multiple protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2, and zucchini yellow mosaic virus. Bio.Tech., 13:1458-1465.

31. Tripathy, Barsha., Pradyumna, Tripathy., Priyanka, Sahu., Sandeep, Rout. and M. Sai Sindhu.(2020). Biofortification of Vegetable Crops - A New Tool to Alleviate Micronutrient Malnutrition. Agriculture and Rural Development: Spatial Issues, Challenges and Approaches.83-97pp. ISBN 978-81-946685-8-9.
32. Tripathy, Barsha., Sandeep, Rout., Lipsa, Dash. and Kalyani, Pradhan.(2020). Micro propagation in vegetable crops: An overview. Times of Agriculture.4:39-42.
33. Yang, L., Shen, H., Pan, A., Chen, J., Huang, C., Zhang, D. (2005). Screening and construct-specific detection methods of transgenic Hufan No 1 tomato by conventional and real-time PCR. J Sci Food Agric 85:2159–2166.
34. Yarra, R., He, S.J., Abbagani, S., Ma, B., Bulle, M., Zhang, W.K. (2012). Overexpression of wheat Na?/H? antiporter gene (TaNHX2) enhances tolerance to salt stress in transgenic tomato plants (*Solanum lycopersicum* L.). Plant Cell Tissue Organ Cult 111:49–57. doi:10.1007/s11240-012-0169-y
35. Zanor, M.I., Osorio, S., Nunes-Nesi, A., Carrari, F., Lohse, M., Usadel, B., Kuhn, C., Bleiss, W., Giavalisco, P., Willmitzer, L., Sulpice, R., Zhou, Y.H., Fernie, A.R. (2009). RNA interference of LIN5 in tomato confirms its role in controlling Brix content, uncovers the influence of sugars on the levels of fruit hormones, and demonstrates the importance of sucrose cleavage for normal fruit development and fertility. Plant Physiol 3:1204–1218.