

# Transforming Fruit Crop Development: Advances in Transgenic Methods

## ABSTRACT

India is second in the world after China in production fruits around the world and these crops play one important role in human diet because they provide Vitamins, minerals, diet fiber and phytochemicals. Flower cultivation in India is considered one of the products for which the demand is constantly increasing, and the export potential of these crops is very high in the global market, but there are problems in the gardening process due to pathogens, pests and weeds, and it requires a large amount of plant protection products per hectare. Genetically modified/GM), Crops enable breeders to bring favorable genes, often already inaccessible, already in elite farming, improving their value significantly and providing unique opportunities to control insects and other pathogens. This novel "molecular farming" provides a very large Possibility, but stringent rules will be required and Control mechanisms to avoid new, potentially serious risks to human health, although possible contamination of food supply. The seed-ambed technology has the potential to reduce pesticide use by increasing plants' resistance to insects. Food security improves by reducing pesticides remain. In addition, Herbicide-Toller Transgenic Crop can help reduce the plow in the fields, so that Save fuel trust for the use of less tractor, whatever it is It reduces the soil structure by reducing its Eater. Transgenic horticulture crops could make important contributions to sustainable horticulture production.

**Keywords:** Genetic modification, abiotic and biotic stress, Vitamins, minerals, diet fiber and phytochemicals

## Introduction

Genetically modified plants are those whose genetic material has been altered in such a way that the quality of the products is increased. This technology is known as recombinant DNA technology or genetic engineering. Genetically modified plants are called transgenic plants. This process of translational integration and expression of a transgene in a plant is called transgenic transformation. Incorporating these traits into the genetic background of a species by conventional breeding requires overcoming some major disadvantages, including long juvenile periods and the incorporation of suitable traits into commercially relevant varieties(Song *et al.*, 2019). However, the current use of new technologies based on high throughput platforms for sequencing and genotyping has contributed profoundly to accelerating the association of these relevant traits with molecular markers and key genes, in addition to those associated with causal factors(Shelton *et al.*, 2017).These species have complex characteristics associated with cross-breeding, such as delayed flowering, failure to set fruit due to embryo destruction, massive fruit drop, and self-incompatibility barriers. Improvement of plant characteristics by transfer of selected genes into the cells of fruit plants is mainly possible through various major methods such as vector-mediated gene transformation, microprojectile bombardment and direct DNA transfer, to generate transgenics for desired characteristics and techniques(Limera *et al.*, 2017, 44-46). which can be used for the production of plants. Foreign DNA can be integrated into cells with new desirable properties. These biotechnological methods improve the genotype of the fruit for important commercial properties such as biotic (disease resistance to viruses, fungi, pests and bacteria) or abiotic (temperature, salinity, light, drought) stress tolerance, nutrition, making it a turn out to be a good product. The choice becomes available and tends to be of quality (fruit ripens later and lasts longer). Among various genetically modified fruit crops, GM papaya shows resistance to papaya ring spot virus, contributing approx 53% of the total share of GM fruit crops cultivated globally. Some phytochemicals derived from fruit crops are strong

antioxidants and are believed to influence processes by protecting against free radical damage, regulating metabolic activation and detoxification of carcinogens, or even altering the course of tumor cells. Play a role in the treatment of chronic disease risk can be reduced. Genetic modification provides a means of adding a desired trait to existing varieties without modifying their commercial characteristics. Fruit tree species face challenges in conventional breeding due to their long generation times and juvenile periods, complex reproductive biology, high levels of heterozygosity, scarce genetic resources and the association of unwanted traits from wild relatives. This ability is especially helpful in these species. Fruit trees have to be genetically improved to improve fruit production. New cultivars created for most of these species take into account horticultural properties related to fertility, yield, attractiveness, quality, disease and pest control, abiotic stress, and shelf-life (Khandelwal *et al.*, 2011).

### **History**

In 1944, the first genetically modified fruit product was placed on the market and regulated in 1992 (Baranski *et al.*, 2019). Fruit softening and shelf life were prolonged in the transgenic fruits due to a decreased expression of a gene that causes pectin solubility. A number of other genetically modified fruit crops are intended to be grown for human consumption or animal feed, and they have been given regulatory approval for commercialization in a number of countries across the world. These include the following: watermelon (*Cucumis melo* L.), pineapple (*Ananas comosus* L.), apple (*Malus domestica* Borkh.), jujube (*Prunus domestica*), tomato (*Solanum lycopersicum*), and papaya (*Carica papaya* L.) (Firoozbady *et al.*, 2015). The majority of transgenic fruits were created with delayed ripening, resistance to pests and diseases, or both, in order to increase agricultural productivity. More recent products, on the other hand, have addressed quality traits by adding new visual traits like pulp color or removing fruit browning. Due to their lack of commercial viability and lack of commercialization, some engineered fruit crops have been removed from the market (Lobato *et al.*, 2021).

### **Advantages of transgenic crop.**

The Breeder Quick technique for agricultural enhancement According to Alvarez *et al.* (2021), developing stable transgenic plants can be completed in three to four years, while traditional breeding methods require twelve to fifteen years to produce a new variety. Break through barrier crossings Gene transfer between unrelated species and even organisms is possible through transgenic breeding. New genotypes evolving through time Transgenic breeding allows for the transfer of genes between different plant species, which can occasionally result in the evolution of new plant species. Consequently, it will have an impact on the natural evolution process. Crop plants, both autogenous and allogamous, can benefit genetically from the application. Success Rate The only genetic improvement of monogenic characters that transgenic breeding is effective for is this. It has been found very effective in developing plants with resistance to various diseases, and insects (Menz *et al.*, 2020).

### **Disadvantages of transgenic crop breeding**

The main drawback of this method is that there is no control over the copy number and side of integration of foreign genes. The high cost of equipment prohibits the use of this method by many researchers for DNA transfer.

### **Mechanism of Transgenic Plant**

There are now many different kinds of plant transformation methods available to the general public. Direct or indirect gene transfer are the two categories into which these plant transformation methods can be divided. While direct gene transfer methods involve the direct

introduction of exogenous DNA into the plant genome through physical or chemical reactions, indirect gene transfer, also referred to as vector-mediated gene transfer, involves the introduction of exogenous DNA into the plant genome through biological vectors.

#### **Plasmid vectors. (Ti plasmid)**

The most widely used vector for creating transgenic plants is the Ti plasmid. Depending on the Ti plasmid class, the estimated size of the plasmid ranges from 200 to 800 kbp. The virulence region, the opine catabolism region, and the transfer DNA (T-DNA) region comprise the three primary regions of the Ti plasmid. About 24 kbp make up the T-DNA segment that is inserted into the plant genome (Baker *et al.*, 1983). Repeat sequences on either end of this region are commonly referred to as the left and right borders. The crucial area required for the transfer of DNA that causes tumorigenesis is the right border. Nonetheless, the virulence region is in charge of encoding the genes, which facilitate the transfer of T-DNA. The biosynthesis of opine and phytohormones (auxin and cytokinin) is also encoded by the T-DNA sequence. The primary causes of plant tumor formation that result in crown gall disease are the three oncogenes (opine, cytokinin, and auxin biosynthesis gene) found in T-DNA (Christie *et al.*, 2014).

#### **Agrobacterium-mediated gene transfer**

The most popular method for transforming plants is called agrobacterium-mediated transformation because it works well with a variety of plants. Innate to the soil ecosystem are agrobacteria. These harmful Gram-negative bacteria are the source of plant hairy root disease and crown gall. These bacteria's genomes contain tumor-inducing plasmids (Ti plasmid) or hairy root-inducing plasmids (Ri plasmid) that carry the genetic material necessary for tumor growth.

#### **Direct gene transfer**

Direct gene transfer refers to the process of introducing exogenous DNA, also known as naked DNA, directly into the nucleus of a plant. The outer membrane of the plant cell must first be broken in order for foreign DNA to enter and penetrate it. The majority of techniques used in direct gene transfer are easy to use and efficient. Nonetheless, these transgenic plants have the ability to change gene expression either permanently or temporarily. There are two primary types of direct gene transfer: chemical gene transfer and physical gene transfer.

#### **Physical gene transfer**

Physical gene transfer uses mechanical techniques to damage the cell membrane and wall. Since Sanford first proposed it, particle bombardment biolistic has been the most often utilized technique for plant transformation. Using a "Gene Gun," high pressure is applied to the target plant cell and the DNA coated with gold or tungsten particles is fired in. The foreign DNA is directed into the nucleus of the plant by the fast-moving particles, which enable coated DNA to pass through the thick cell wall. After that, the coated DNA will detach from the metal particles and incorporate itself into the plant cell's nucleus' chromosomes (Lai *et al.*, 1997).

#### **Chemical gene transfer**

The most common chemical used in chemical gene transfer approaches is polyethylene glycol (PEG), which is used to destabilize the cell membrane in the presence of a divalent cation, increasing the permeability of the cell membrane and allowing for the uptake of foreign DNA. The exact mechanism for chemical gene transfer is not fully understood, but it was postulated that PEG increases the osmotic pressure and causes contraction in the protoplast; this facilitates endocytosis of the divalent cation/DNA complex (Lazzeri *et al.*, 1991). In addition to those, a different chemical technique called liposomes is

employed to change the protoplast cells of plants. To encapsulate and transfer foreign genetic materials into the protoplast, liposomes are used. Because liposomes are lipophilic, they can easily enter the protoplast and transform the cell (Caboche *et al.*, 1990).

### Use of Transgenic Approach in Fruit Crops

Crop	Character	Gene transferred	Method of gene Transfer	Variety	Reference
<b>Transgenic papaya</b>	PSRV Resistant	coat protein gene from PRSV	micro projectile bombardment technique	Sun UP from Sunset and UH Rainbow from Kapoho	(Gonsalves 1992)
<b>Transgenic banana</b>	Banana Bunchy top Virus	Replicase-associated gene (Rep gene)	RNAi technology <i>Agrobacterium</i> mediated transformation	Dwarf Brazilian (AAB) Pome sub group	(Borth <i>et al.</i> , 2011)
		master replication initiation protein (Rep)	<i>Agrobacterium</i> mediated transformation	Rasthali' (AAB genome)	(Shekhawat <i>et al.</i> , 2012)
		Replicase-associated gene (Rep gene) RGA2 gene from banana and Ced9 gene, is derived from a nematode	<i>Agrobacterium</i> mediated transformation	Virupakshi (AAB)	(Elayabalan <i>et al.</i> , 2013)
	<i>Xanthomonas</i> wilt	Plant ferredoxin-like protein ( <i>Pflp</i> ) gene from sweet pepper ( <i>Capsicum annuum</i> ).	<i>Agrobacterium</i> mediated transformation	'SukaliNdiizi', and 'Nakinyika',	(Klopez, 2012)
<b>Transgenic Pear</b>	Fire blight ( <i>Erwinia amylovora</i> ) produces (desferrioxamine protein	Exogenous ferritin gene which acts as iron chelator from pea	„	'Conference' and 'Passe-Grassane'	(Djennane <i>et al.</i> , 2009)
<b>Grapefruit (<i>Citrus paradisi</i>)</b>	citrus tristeza	closterovirus genes	„	-	(Febres <i>et al.</i> , 2008)

<b>Trifoliolate orange</b>	salinity tolerance	Betaine aldehyde dehydrogenase gene ( <i>AhBADH</i> ) gene cloned from <i>Atriplex hortensis</i>	„	-	(Fu <i>et al.</i> , 2011)
<b>Guava (<i>Psidium guajava</i>)</b>	guava wilt	Trichoderma-endochitinase gene	„	-	(Mishra <i>et al.</i> , 2014)
	cold temperatures tolerance	cold hardiness genes (CBF1, CBF2 and CBF3)	„	-	(Mishra <i>et al.</i> , 2014)
<b>Kiwifruit</b>	Resistance to the insect <i>Oraesia excavate</i>	synthetic chimeric gene SbtCry1Ac that encodes the insecticidal protein btCry1Ac	„	-	(Fu <i>et al.</i> , 2011)
<b><i>Vitis vinifera</i></b>	Virus resistance	CP of ArMV	Somatic embryos	-	(Spielmann <i>et al.</i> , 2000)
	Fungal resistance	Rice chitinase (RCC2)	Somatic embryos	‘NeoMuscat’	Yamamoto <i>et al.</i> , 2000)
	Cold resistance	SOD from Arabidopsis	<i>A. tumefaciens</i>	‘Cabernet Franc’	(Rojas <i>et al.</i> , 1996)
	Modified fruit traits	DefH9/iaaM	<i>A. tumefaciens</i>	Silcora’, ‘Thompson Seedless’	(Mezzetti <i>et al.</i> , 2002)
Apple	Non browning	PPO suppression transgene, ACC oxidase, ACC synthase	<i>Agrobacterium rhizogenes</i>	Golden Delicious, Fuji, Gala	(Zhu <i>et al.</i> , 2001)
	Resistance to fire blight	NPTII attE, nptII, gusA	Replicase associated protein	Gala	(Kost <i>et al.</i> , 2015)
Strawberry	Powdery mildew	Chitinase, rice PpMlo1	<i>A. tumefaciens</i>	-	(Jiwan <i>et al.</i> , 2013)
Pear	Fire blight	Cecropin genes SB-37 and Shiva-1 and Attacin E	<i>A. tumefaciens</i>	-	(Malnoy <i>et al.</i> , 2005)
Plum	Plum pox virus	Coat protein, plum pox virus	<i>A. tumefaciens</i>	-	(Malinowski <i>et al.</i> , 2006)

### Future Prospects

Improved fruit varieties can now be produced with new tools thanks to genetic engineering advancements, especially the emergence of genome editing techniques. Numerous fruit crop proof-of-concept cases have been documented, and the continued advancement and promotion of these cultivars may have a significant socioeconomic influence. Even though transgenic research in many fruit crops has advanced significantly,

more work needs to be done before the transgenic approach becomes the go-to method for fruit improvement. In order to control genome-edited plants and their products, a number of nations have modified their existing laws or created new ones in recent years. This could mean that in nations where genome editing is permitted, fruits with altered genomes could become available sooner than all other edited crops. The fruit varieties that have already received approval for commercialization are covered here, with an emphasis on those that are currently available. Next, we delve into the socioeconomic implications of fruit varieties that have been created recently through the use of transgenic fruit crops. Significant issues that must be addressed are consumer acceptability and environmental safety. Finding fresh and improved candidate genes for various traits within the same species and/or genus will be helpful. The anticipated impact of manipulating target genes on fruit improvement programs is expected to be significant.

## Conclusion

From above the discussion, it is concluded as genetically-modified fruit crops have the potential to solve many of the world's hunger and malnutrition problems. According to many researchers it concludes that transgenic techniques help to improve in the genetically development of fruit crops by gene transfer. There is no doubt that transgene removal will eventually be routine as some of the techniques reviewed here are perfected. The accumulated information derived from this scaling-up process fused to the characterization of some of the kinetic parameters involved in grapevine SE, have enabled design of new experimentation focused on the development of SE protocols for genotypes cultivars such as 'Red Globe'. The results indicate that grape genetic transformation can be considered as a model system in which efficiency is not necessarily an issue and the possibility for high through-put candidate gene evaluation is plausible.

## References

1. Alvarez, D., Cerda-Bennasser, P., Stowe, E., Ramirez-Torres, F., Capell, T., Dhingra, A., & Christou, P. (2021). Fruit crops in the era of genome editing: closing the regulatory gap. *Plant cell reports*, 40, 915-930.
2. Baranski, R., Klimek-Chodacka, M. & Lukasiewicz, A. Approved genetically modified (GM) horticultural plants: a 25-year perspective. *Folia Horti*. **31**, 3–49 (2019).
3. Barker RF, Idler KB, Thompson DV, Kemp JD 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Molecular Bio-logy*. **2**(6):335-350.
4. Borth, W., Perez, K., Cheah, Y., Chen, W. S., Xie, D., Gaskill, S., Khalil, D., Sether, M., Melzer, M., Wang, R., Manshardt, D., Gonsalves and Hu, J.S.2011. Transgenic banana plants resistant to banana bunchy top virus infection. *Acta Hort*. 897:449-457.
5. Bruening, G., & Lyons, J. (2000). The case of the FLAVR SAVR tomato. *California Agriculture*, 54(4)6-7.
6. Caboche M. (1990) Liposome-mediated transfer of nucleic acids in plant protoplasts. *Phy-siologia Plantarum*.**79**(1):173-176.
7. Chiera, J, M, Bouchard, R,A., Dorsey SL, Park E, Buenrostro-Nava MT, Ling PP. 2007. Isolation of two highly active soybean (*Glycine max* (L.) Merr.) promoters and their characterization using a new automated image collection and analysis system. *Plant Cell Reports*.**26**(9):1501-1509.
8. Christie PJ, Gordon JE.2014. The *Agrobacterium* Ti plasmids. *Microbiology Spectrum*. **2**(6):295-313.

9. Djennane, S, Cesbron, C, Sourice, Sophie and Loridon K and Chevreau, E. 2009. Production of transgenic pear plants expressing ferritin gene with aim to reduce fire blight susceptibility. *Acta Horticulturae*. **814**:781-786.
10. Elayabalan, S. K., Kalaiponmani, S., Sreeramanan, R., Selvarajan, P., Radha, M., Ramlatha, K., Kumar, K and Balasubramanian, P. 2013. Development of Agrobacterium mediated transformation of highly valued hill banana cultivar Virupakshi (AAB) for resistance to BBTv disease. *World J. Microb. Biotech.* **29**:589-596.
11. Febres, V.J., Lee, R.F and Moore, G.A. 2008. Transgenic resistance to Citrus tristeza virus in grapefruit. *Plant Cell Reports*. **27**: 93-104.
12. Firoozbady, E., & Young, T. R. (2015). *U.S. Patent, 13 / 507 : 101*.
13. Fu, X., Khan, E.U, Hu, S.S., Fan, Q.J., Liu, J.H. 2011. Overexpression of the betaine aldehyde dehydrogenase gene from *Atriplex hortensis* enhances salt tolerance in the transgenic trifoliate orange (*Poncirus trifoliate* L. Raf). *Environmental and Experimental Botany* **74**: 106-113.
14. Gonsalves C, Tennant P, Fermin G, Souza M. 1992. A protocol for efficient transformation and regeneration of *Carica papaya* L, In Vitro Cellular and Developmental Biology. *Plant* **35**: 61-69.
15. Hernandez-Garcia CM, Martinelli AP, Bouchard RA, Finer JJ. 2009. A soybean (*Glycine max*) polyubiquitin promoter gives strong constitutive expression in transgenic soybean. *Plant Cell Reports*. **28**(5):837-849.
16. Ihemere U, Arias-Garzon D, Lawrence S, Sayre R. 2006. Genetic modification of cassava for enhanced starch production. *Plant Biotechnology Journal*. **4**(4):453-465.
17. Jiwan, D., Roalson, E.H., Main, D., Dhingra, A., 2013. Antisense expression of peach mildew resistance locus O (*PpMlo1*) gene confers cross-species resistance to powdery mildew in *Fragaria x ananassa*. *Transgenic Res.* **22**, 1119–1131.
18. Karaaslan M, Hrazdina G. 2010. Characterization of an expansin gene and its ripening-specific promoter fragments from sour cherry (*Prunus cerasus* L.) cultivars. *Acta Physiologiae Plantarum*. **32**(6):1073-1084.
19. Kawakatsu T, Takaiwa F. 2010. Cereal seed storage protein synthesis: Fundamental processes for recombinant protein production in cereal grains. *Plant Biotechnology Journal*. **8**(9):939-953.
20. Khandelwal, Kuruganti, K. and Ramanjaneyulu, G.V. 2011. Genetic Engineering in Indian Agriculture - An Introductory Handbook: 2-5.
21. Klopez. 2012. Transgenics in crop improvement research, IITA, accessed from <http://r4dreview.iita.org/index.php/2011/04/14/transgenic-banana-forafrica/> on 25-9-2018.
22. Kost, T.D., Gessler, C., Jansch, M., Flachowsky, H., Patocchi, A., Broggini, G.A.L., 2015. Development of the first cisgenic apple with increased resistance to fire blight. *PLoS One* **10**, e0143980.
23. Lai KS, Abdullah P, Yusoff K, Mahmood M. 2011. An efficient protocol for particle bombardment-mediated transformation of *Centella asiatica* callus. *Acta Physiologiae Plantarum*. **33**(6):2547-2552.
24. Lai KS, Masatsugu T. 2013. Isolation and characterization of an *Arabidopsis thaliana* self-incompatibility mutant induced by heavy-ion beam irradiation. *Acta Biologica Craco-viensia/Series Botanica*. **55**(2):146-152.
25. Lai KS. 2016. Analysis of EXO70C2 expression revealed its specific association with late stages of pollen development. *Plant Cell, Tissue and Organ Culture*. **124**(1):209-215.

26. Lazzeri PA, Brettschneider R, Lührs R, Lörz H. (1991). Stable transformation of barley via PEG-induced direct DNA uptake into protoplasts. *Theoretical and Applied Genetics*. **81**(4):437-444.
27. Lim YY, Lai KS. 2017. Generation of transgenic rice expressing cyclotide precursor *Oldenlandia affinis* kalata B1 proetin. *Journal of Applied Pharmaceutical Science*. **27**(2):667-671.
28. Limerá, C., Sabbadini, S., Sweet, J. B., Mezzetti, B. & Kühn-institut, J. New biotechnological tools for the genetic improvement of major woody fruit species. *Front. Microbiol.* **8**, 1–16 (2017).
29. Lobato-Gómez, M., Hewitt, S., Capell, T., Christou, P., Dhingra, A., & Girón-Calva, P. S. (2021). Transgenic and genome-edited fruits: background, constraints, benefits, and commercial opportunities. *Horticulture Research*, **8**, 166.
30. Malinowski, T., Cambra, M., Capote, N., Zawadzka, B., Gorris, M.T., Scorza, R., Ravelonandro, M., 2006. Field trials of plum clones transformed with the *Plum pox virus* coat protein (PPV-CP) gene. *Plant Dis.* **90**, 1012–1018.
31. Malnoy, M., Faize, M., Venisse, J.S., Geider, K., Chevreau, E., 2005. Expression of viral EPS-depolymerase reduces fire blight susceptibility in transgenic pear. *Plant Cell Rep.* **23**, 632–638.
32. Mezzetti, B., Pandolfini, T., Navacchi, O., Landi, L. 2002. Genetic transformation of *Vitis vinifera* via organogenesis. *BMC Biotechnology*. **2**:18.
33. Mishra, M., Jalil, S.U., Sharma, N., Hudedaman, I. U. 2014. An Agrobacterium mediated transformation system of guava (*Psidium guajava* L.) with endochitinase gene. *Crop Breeding and Applied Biotechnology*. **14**: 232-237.
34. Navarre C, Sallets A, Gauthy E, Maîtrejean M, Magy B, Nader J.2011. Isolation of heat shock-induced *Nicotiana tabacum* transcription promoters and their potential as a tool for plant research and biotechnology. *Transgenic Research*.**20**(4):799-810.
35. Odell JT, Nagy F, Chua NH. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature*. **313**(6005):810-812.
36. Rojas, B., McKersie, B. D, Paroschy, J.H. 1996. Agrobacterium-mediated transformation of *Vitis vinifera*. 4<sup>th</sup> Canadian Plant Tissue Culture and Genetic Engineering Workshop, Saskatoon
37. Shekhawat, U. K. S., R. Thumballi, T. R. Ganapathi and A. B. Hadapad. 2012. Transgenic banana plants expressing small interfering RNAs targeted viral replication initiation gene display high-level resistance to banana bunchy top virus infection. *J. Gen. Virol.* **93**:1804-1813.
38. Shelton, A. M., Hokanson, K. E., Hautea, D. M., Hossain, M. J., Hossain, M. A., Paranjape, V. and Sarwer, S. H. (2017). Bt Eggplant: a genetically engineered ‘minor’ crop comes of age in Bangladesh and the Philippines. *ISB News Report*.
39. Sivamani E, Qu R. 2006. Expression enhancement of a rice polyubiquitin gene promoter. *Plant Molecular Biology*. **60**(2):225-239.
40. Song, G., Prieto, H. & Orbovic, V. Agrobacterium-mediated transformation of tree fruit crops: methods, progress, and challenges. *Front. Plant Sci.* **10**, 226 (2019).
41. Spielmann, A., Krastanova S, Douet-Orhant V, Gugerli, P.2000. Analysis of transgenic grapevine (*Vitisrupestris*) and *Nicotiana benthamiana* plants expressing an *Arabis* mosaic virus coat protein gene. *Plant Science***156**: 235-244.
42. Yamamoto, T., Iketani, H., Ieki, H., Nishizawa, Y., Notsuka, K., Hibi T., Hayashi, T., Matsuta, N. 2000. Transgenic grapevine plants expressing a rice chitinase with enhanced resistance to fungal pathogens. *Plant Cell Reports* **19**:639- 646.

43. Zhu, L.-H., Holfors, A., Ahlman, A., Xue, Z.-T., Welander, M., 2001. Transformation of the apple rootstock M.9/29 with the rolB gene and its influence on rooting and growth. *Plant Sci.* **160**: 433–439.
44. Kaur H, Talekar N. The Function of RNA Interference (RNAi) in Crop Enhancement-Mechanism and Applications: A Review. *J. Exp. Agric. Int.* [Internet]. 2024 May 8 [cited 2024 May 17];46(6):351-70. Available from: <https://journaljeai.com/index.php/JEAI/article/view/2487>
45. Anand KJ, Nagre SP, Shrivastava MK, Amrate PK, Patel T, Katara VK. Enhancing Crop Improvement through Synergistic Integration of Advanced Plant Breeding and Proximal Remote Sensing Techniques: A Review. *Int. J. Plant Soil Sci.* [Internet]. 2023 Aug. 16 [cited 2024 May 17];35(19):121-38. Available from: <https://journalijpss.com/index.php/IJPSS/article/view/3533>
46. Kamthan A, Chaudhuri A, Kamthan M, Datta A. Genetically modified (GM) crops: milestones and new advances in crop improvement. *Theoretical and Applied Genetics.* 2016 Sep;129:1639-55.
- 47.