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TRANSGENIC TECHNIQUE FOR GENETIC IMPROVEMENT IN FRUITS CROPS

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Abstract: *Fruits play a pivotal role for they provide vitamins, minerals, dietary fibre, and phytochemicals to human diet. Its consumption worldwide is rising, reflecting the consumer's increased income, desire of diversity and awareness of nutritional benefits. Fruit crops production suffers from many biotic stresses caused by pathogens, pests and weeds and involves the usage of high amounts of plant protection chemicals per hectare. These pesticide residues can affect the health of growers and consumers as well as contaminate the environment. Hence developing resistant varieties of fruit crops to different biotic and abiotic stress is of greatest concern. Progress in traditional plant breeding is limited by the genetic diversity within each crop species, the diversity sometimes available from closely related species or occasionally useful diversity created within the crop itself by inducing mutations. Often, genes of interested traits could not be found in a particular crop species, so the new desirable traits borrowed from other species represents a major technological advance over conventional breeding methods. Genetically-modified fruit crops in addition to food grain crops have the potential to solve many of the world's hunger and malnutrition problems and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Transgenic research in fruit crops is still in its infancy. However, available information can be utilized by fruit breeders to design future program for developing transgenic that ultimately open the way for those crops where gene transfer is difficult through conventional breeding.*

Keywords: *Fruits, vitamins, minerals, dietary fibre, diet and phytochemicals.*

Introduction: Fruits are grown worldwide and make up a major portion of the diet of humans in many parts of the world. They play a significant role in human nutrition, especially as sources of vitamins (C, A, B1, B6, B9,E), minerals, dietary fiber and phytochemicals [1]. Vegetables and fruits in the daily diet are strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer [2]. Fruit consumption worldwide is rising, reflecting the consumer's increased income, desire of diversity and awareness of nutritional benefits. Fruit and vegetables production, due to their cultivation intensity, suffers from many biotic stresses caused bypathogens, pests and weeds and requires high amounts of pesticides per hectare. The main method for controlling pathogens, pests, and weeds has been the use of pesticides because vegetables and fruits are high-value commodities with high cosmetic standards. Insecticides are regularly applied to control a complex of insect pests that cause damage by feeding directly on the plant or by transmitting pathogens, particularly viruses. Despite pesticide use, insects, pathogens, and weeds continue to cause a heavy toll on world vegetable and fruit production. Pesticide residues can affect the health of growers and consumers and contaminate the environment. Consumers worldwide are increasingly concerned about the quality and safety of their food, as well as the social and the environmental conditions under which it is produced [1]. Hence developing resistant varieties of fruit crops to different biotic and abiotic stress is of greatest concern. Progress in traditional plant breeding is limited by the genetic diversity within each crop species, the diversity sometimes available from closely related species or occasionally useful diversity created within the crop itself by inducing mutations. Often, genes of interested traits could not be found in a particular crop species, so the new desirable traits borrowed from other species represents a major technological advance over conventional breeding methods [3]. During the last two–three decades, genetic engineering methods based on the use of transgenes have been successfully adopted to improve fruit plants and focused mainly on enhanced tolerance to biotic and abiotic stresses, increased fruit yield, improved post harvest shelf life of fruit, reduced generation time and production of fruit with higher nutritional value.

Main Features of Transgenic Plants: Genes can now be transferred into plants from a wide range of organisms, including unrelated plant species, microbes, animals, and from DNA synthesized from laboratory [4]. In the year ahead, transgenic variety will be produced that have modification in a wide range of characters and have genetic changes not achievable through the conventional breeding methods. The features of transgenic plants are briefly discussed below [5]:

1. Contain Transgenes: Transgenic plant contains transgene (foreign gene). The foreign gene may be utilized fromunrelated plant, microbes and animal. Microbial genes are utilized from fungi, bacteria and viruses. Sometimes, genes from DNA synthesized in the laboratory are also used for development of transgenic plants.

2. Involved Biotechnology: Development of transgenic plants involves plant biotechnology, which refer to the combination of tissue culture and genetic engineering. The foreign gene cannot be inserted into whole plants. It can only be inserted into single cells (tissue culture). Thus combination of tissue culture and molecular genetics is essential for development of transgenic plants.

3. Bypass Sexual Process: In the development of transgenic plants, the sexual process is bypassed. Transgenic plants are developed without involving sexual fusion between donor and the recipient parents. Once a transgenic plant is developed, the transgenic trait can be transferred to other genotypes through backcross method.

4. Low Frequency: In most of the fruit crops, transgenic plants are recovered at a very low frequency. Hence, huge single cells or protoplasts have to be screened in the culture media for identification and isolation of transgenic cells. The transformed cells are identified by Polymerase Chain Reaction (PCR) technique.

5. Utility: Transgenic plants are developed to solve specific problems in crop plants such as development of plants resistance to diseases, insects, drought, frost, salinity and metal toxicity, improvement of keeping quality and development of male sterility, *etc.*

6. Rapid Method of Crop Improvement: Stable transgenic plant can be developed in 3-4 years, whereas it takes 12-15 years to develop a new variety through conventional methods of breeding.

7. Overcome Crossing Barriers: Transgenic breeding permits gene transfer between unrelated species and even between unrelated organisms. For example, a freezing resistant gene has transfer from fish to cultivated tomatoes. Similarly, ovalbumin gene of chicken has been transfer in alfalfa for improving protein quality.

8. Evolution of New Genotypes: Sometimes transgenic breeding may lead to evolution of altogether new plant species, because it permits gene transfer between various plant species. Thus, it will affect the process of natural evolution.

9. Application: It can be used for the genetic improvement of both autogamous and allogamous crop plants.

10. Effectiveness: Transgenic breeding has been found effective for the genetic improvement of monogenic characters only. Both seed and vegetative propagated species can be improved through the use of transgenes.

Some Achievements in Fruit Crops by Transgenic Technique

Papaya: Resistance to Papaya Ringspot Virus (PRSV): *Papaya ringspot virus* (PRSV) reduces both fruit quality and edible yield. A transgenic approach could be used to introduce traits to provide resistance to important pests. Transgenic papaya with pathogen derived resistance carrying the coat-protein gene provides effective protection against PRSV strains over a significant period of the cycle of this perennial fruit crop [6]. Seeds of the transgenic cultivars “SunUp” and “Rainbow” became available to Hawaii’s farmers in 1998 and growing cultivar “Raibow” led to a significant increase in papaya production [7]. The cultivation of PRSV-resistant transgenic papaya cultivars dramatically reduced the incidence of PRSV in many areas. Growers have been able to use the PRSV-resistant transgenic papaya cultivars as a trap crop [8] by growing it as a border around the non-transgenic crop and allowing it to cleanse viruliferous aphids of PRSV [9]. Thus, the Hawaiian papaya industry can now produce and market both transgenic and conventional papaya in the same field.

Plum: Resistance to *Plum pox virus* (PPV): *Plum pox virus* (PPV) is one of the worldwide most destructive diseases of plum and other stone fruits. Over the past 20 years an intensive international research project between USA, France, Poland, Romania, Spain and Czech Republic has focused on the development of transgenic resistance to PPV [10]. The PPV coat protein (CP) gene was isolated, sequenced and cloned [11] and was used for *Agrobacterium*-mediated transformation of plum. As a result, the highly PPV-resistant cultivar "Honey Sweet" plum was bred. This cultivar has shown its host plant resistance after 15 years of field testing in Europe underheavy infection pressure [12-13] and in greenhouse tests in the USA. Its transgenic resistance was further transferred to other seedlings through crossbreeding [14-15], thus developing additional PPV-resistant cultivars. The inheritance pattern of PPV resistance is transferred to the offspringas a single dominant gene. Studies show that "Honey Sweet" plum fruit quality is excellent, and productivity is very good [15]. "Honey Sweet" plum provides an interesting germplasm source for PPV control elsewhere. The transgene resistance technology and post-transcriptional gene silencing tested in "Honey Sweet" can be used to develop other resistant stone fruits, such as peach, apricot, Japanese plum and cherry, which are susceptible to PPV.

Banana: Resistance to *Xanthomonas* Wilt: Banana and plantain (*Musa* sp.) are among the most important food crops in the tropics. *Xanthomonas* wilt caused by the bacterium *Xanthomonascampestris* pv. *musacearum*is one of the most important diseases and currently considered as the biggest threat to banana production in the Great Lakes Region of Africa including Uganda, Democratic Republic of Congo, Rwanda, Kenya, Tanzania and Burundi [3]. The pathogen is highly contagious andits spread has endangered the live hood of millions of farmers who rely on banana for food and income. The International Institute of Tropical Agriculture (IITA), in a partnership with Uganda's National Agricultural Research Organisation(NARO) and other institutions have developed transgenic bananas constitutively expressing the hyper sensitivity response-assisting protein (*Hrap*) and the plant ferredoxin-like protein (*Pflp*) genes from sweet pepper (*Capsicum annuum*). These transgenic bananas have exhibited enhanced resistance to *X. campestris* pv. *Musacearum* [16]. Transgenic bananas did not show significant changes in plant morphology and the bunch weight and size versus the non-transgenic banana cultivar. They will be further evaluated for environmental and food safety according to the country's biosafety regulations, risk assessment and management, plus procedures for seed registration and release [17].

Banana: Edible Vaccines: Transgenic banana is one of the recent examples, where genes of antigenic protein of many deadly disease causing pathogens have been expressed in banana fruits. Thus children can be immunized only by feeding with this transgenic fruits, instead of painful injections for immunization.

Pomegranate: Resistance to Bacterial Blight Disease: Pomegranate production in India is severely hampered by the high incidence of bacterial blight disease caused by *Xanthomonasaxonopodis* pv. *punicae* is air borne. Evolving a resistant genotype using resistant variety through conventional breeding may be a way out but it is a time consuming process. One of the strategies to lead plants become resistant against bacterial pathogens is employing a transgene, like plant ferredoxin-like protein (PFLP). Different treatment combinations of hormonal concentrations were taken for leaf, petal, nodes and cotyledonary explants to standardize an efficient *in vitro* regeneration protocol and find out the best treatment for faster regeneration. *Agrobacterium tumefaciens*carrying gene pCAMBIA construct with the constitutive CaMV35S promoter, PFLP gene, terminator and *nptII* selectable marker (Kanamycin resistance), was used for transformation of explants [18]. Putative transformants were identified on selection medium containing kanamycin at different concentration. Integration of transgene and expression at various levels were confirmed using PCR.

Mulberry: **Resistance to Abiotic Stress:** Abiotic stresses remain the greatest constraint to crop production. Genetic engineering was adopted to enhance drought and salt tolerance in mulberry using HVA1 and *Osmotin genes*. Generated transgenic mulberryexpressing GUS genes [19]. In the process, it is foundthat A. *tumefacien*s train LBA4404 was more infective than the other strains. Nearly 90-100%transformation was obtained with LBA4404, 70-75% with GV2260 and 25-35% with A281. Among the plasmids, pBI121 and pBI101:Act1 were most efficient (100%) [20]. Subsequently, several transgenicmulberry plants overexpressing desired genes were developed, including glycine gene *Ala Blb*, the oryzacystatin gene *OC* and the barley *HVA1* gene [21-23]. The transgenic mulberryover-expressing *HVA1* from barley show edcomparatively better growth under abiotic stresses [22-23]. The transgenicplants with *Osmotin*gene under the constitutive expression of the CaMV 35S promoter and a stress inducible promoter *rd29A* also expressed high salt, drought and cold stress tolerance [1].

Pineapple: Resistance to Herbicide: Transgenic pineapple plants transformed with the bar gene for bialaphos resistance were developed [24] and evaluated for tolerance to herbicide Basta. The effect of Basta2 concentrations in excess of 3mg lÿ1glufosinate ammonium on non-transformed plants was observed within 4 days of herbicide application, with inhibition of growth and loss of pigmentation and died rapidly after 7 days, whereas all PPT-resistant plants and their clones remained green. Transgenic plants tolerant to glufosinate ammonium should facilitatemore effective weed control in pineapple plantations without damage to the crop [25].

Apple: Resistance to Browning: The "non browning" apple (*Malus x domestica*) is genetically engineered to prevent browning after being cut. When apple flesh is cut and exposed to oxygen, it begins to brown. But the genetically modified apple or "Arctic Apple," is resistant to browning. The genetically modified apples are designed to look fresh when they're not. It was developed by silencing a gene in the apple (that controls browning) by inserting modified apple DNA. It was approved for sale by USDA in 2015.

Grape: Higher Yield: Conducted an experiment to know the fecundity and total fruit production of Thompson seedless (TS) and Silcora (S) Def H9iaam lines (GM) in comparison with control [26]. Thompson Seedless DefH9-iaaM line had twice as many bunches per plant than its control. The increase in bunch number depends on the increased number of inflorescences per shoot detected in the transgenic line. In the transgenic Thompson Seedless line, the number of berries per bunch was30% more and average bunch weight is 25% more than its control. Although the average berries weight was slightly lower in the DefH9-iaaM Thompson Seedless line than the control.

Strawberry: Higher Fruit Firmness: Conducted an experiment to know external and internal firmness in controls and transgenic strawberry Apel plants [27]. The main differences between Apel and control fruits were observed in firmness. Fifty percent of the Apel clones analyzed showed a higher external fruit firmness than fruits obtained from control plants. It is noteworthy that most of the Apel lines displayed a statistically significant increment in the internal fruit firmness (obtained after removing the external skin of the fruit), ranging the increment from 149% to a 179% when compared with macro propagated plants.

Conclusion: Fruits are the rich sources of vitamins and the other nutrients, these sources can be improved by the means of this transgenic breeding. Genetically modified crops offer dramatic promise for meeting the great challenges of twenty first century. Transgenic breeding help us for trait specific breeding, it can be a tool to increase productivity. Furthermore, transgenic crop provide resistance against ring spot virus in papaya, plum pox virus in plum, bacterial blight in pomegranate, which contributes to integrated pest management in horticulture by reducing pesticide sprays as well as improving food safety by minimizing pesticide residues. herbicide-toleranttransgenic crops can help reducing plough in fields which protects the structure of the soil by reducing soil erosion.

References

1. Das, M., Chauhan, H., Chhibbar, A., Haq, Q. M. R. and Khurana, P. (2011). High-efficiency transformation and selective tolerance against biotic and abiotic stress in mulberry, *Morusindica*cv. K2, by constitutive and inducible expression of tobacco osmotin.*Transgenic Res.*, 20: 231-246.
2. Keatinge, J.D.H., Waliyar, F., Jammadass, R.H., Moustafa, A., Andrade, M., Drechsel, P., Hughes, J.A., Kardivel, P. and Luther. K. (2010). Re-Learning Old Lessons for the Future of Food: By Bread Alone No Longer-Diversifying Diets with Fruit and Vegetables. *Crop Science*, 50: 51-62.

3. Dias, J.S. and Ortiz, R. (2014). Advances in transgenic vegetable and fruit breeding. *Agricultural Sciences*, 5: 1448-1467.
4. Kumar, S., Kakraliya, S.K., Lakhran H., Sheoran, S., Ram, M.,and Meena, R.S. (2016). Use of *Bacillusthuringiensis* (*Bt*) gene: A biotechnological tool for crop improvement, *Progressive Research – An International Journal*, 11 (Special-IX): 6247-6251.
5. Tanuja, P. and Kumar, A.L. (2017). Transgenic fruit crops – A Review. *Int. J.Curr. Microbiol. App. Sci*, 6(8): 2030-2037.
6. Lius, S., Manshardt, R.M., Fitch, M.M.M., Slightom, J.L., Sanford, J.C. and Gonsalves, D. (1997). Pathogen derived resistance provides papaya with effective protection against papaya ringspot virus. *Molecular Breeding*, 3: 161-168
7. Susuki, Y.I., Tripathi, S. and Gonsalves, D. (2007). Virus resistant transgenic papaya: Commercial Development and Regulatory and Environmental Issues. In: Punka, Z.K., De Boer, S.H. and Sanfacon, H., Eds., *Biotechnology and PlantDisease Mmanagement*, CAB International, Wallinford, 436-461.
8. Shelton, A.M. and Badenes-Perez, F.R. (2006). Concept and applications of trap cropping in pest management. *Annual Review of Entomology*, 51: 285-308.
9. Fuchs, M. and Gonsalves, D. (2007). Safety of virus resistant transgenic plants two decades after their introduction: Lessons from Realistic Field Risk Assessment Studies. *Annual Review of Phytopathology*, 45: 173-202.
10. Scorza, R., Callahan, A., Dardick, C., Cambra, M., Polak, J., Ravelonandro, M., Zagrai, I. and Malinowski, T. (1998). "Honey Sweet"-A transgenic Plum Pox Virus resistant plum-from laboratory and experimental field plots to regulatory approval. *Acta Horticulturae*, 974: 57-63.
11. Ravelonandro, M., Monsion, M., Teycheney, P.Y., Delbos, R. and Dunez, J. (1992). Construction of a chimeric viral gene expressing Plum Pox Virus coat protein. *Gene*, 120: 167-173.
12. Hily, J.M., Scorza, R., Malinowski, T., Zawadzka, B. and Ravelonandro, M. (2004). Stability of gene silencing-based resistance to Plum Pox Virus in transgenic plum (*Prunusdomestica*L.) under field conditions.*Transgenic Research*, 13: 427-436.
13. Polak, J., Pivalova, J., Kundu, J.K., Jokes, M., Scorza, R. and Ravelonandro, M. (2008). Behavior of transgenic Plum Pox Virus-resistant *Prunusdomestica*L. clone C5 grown in the open field under a high and permanent infection pressure of the PPV-Rec Strain. *Journal of Plant Pathology*, 90, S1.33-S1.36.
14. Ravelonandro, M., Scorza, R., Renaud, R. and Salesses, G. (1998). Transgenic plums resistant to Plum Pox Virus infection and preliminary results of cross-hybridization. *Acta Horticulturae*, 478: 67-71.
15. [b]Scorza, R., Callahan, A., Levy, L., Damsteegt, V. and Ravelonandro, M. (1998). Transferring Potyvirus coat protein genes through hybridization of transgenic plants to produce Plum Pox Virus resistant plums (*Prunusdomestica*L.). *ActaHorticulturae*, 472, 421-425.
16. Tripathi, L., Mwaka, H., Tripathi, J.N. and Tushemereirwe, W. (2010). Expression of sweet pepper *Hrap*gene in banana enhances resistance to *Xanthomonascampestris*pv*musacearum*. *Molecular Plant Pathology*, 11: 721-731.
17. Tripathi, L. (2012). Transgenics in crop improvement research at IITA. *IITA Research for Development* (*R4D*) *Review*, 8: 58-60.
18. Hosamani, A., Mohandas, S., Manjula, V., Kerure, P. and Geetha, M.S. (2016). *Pflp*gene transformation in pomegranate (*Punicagranatum*L.) resistance to bacterial blight disease (*Xanthomonasaxonopodis*pv. *punicae*).*Electronic Journal of Plant Breeding*, 7(4): 864-870.
19. Bhatnagar, S and P. Khurana. (2003). *Agrobacterium* tumefaciens-mediated transformation of Indian mulberry, *Morusindica*cv. K2: A time phased screening strategy. *Plant Cell Rep*., 21:669-675.
20. Vijayan, K., Raju, P.J., Tikader, A. and Saratchnadra,B. 2014.Biotechnology of mulberry (*Morus*L.) - A review.*J. Food Agric*., 26(6): 472-496.
21. Wang, H., Lou, C., Zhang, Y., Tan, J. and Jiao, F. (2003). Prelimarily report on oryzacystatin gene transferring into mulberry and production of transgenic plants. *Acta Sericol. Sinica*, 29:291-294.
22. Lal, S., Gulyani, V. and Khurana, P. (2008). Overexpression of HVA1 gene from barley generates tolerance to salinity and water stress in transgenic mulberry (*Morusindica*). *Trans. Res.*, 17: 651-663.
23. Checker, V.G., Chhibbar, A.K. and Khurana, P. (2012). Stress-inducible expression of barley Hva1 gene in transgenic mulberry displays enhanced tolerance against drought, salinity and cold stress. *Transgenic Res*., 21: 939-57.
24. Sripaoraya, S., Marchant, R., Power, J.B. and Davey, M.R. (2001). Herbicide-tolerant transgenic pineapple (*Ananascomosus*) produced by micro projectile bombardment. *Annals of Botany*, 88: 597-603.
25. Thakur, A.K., Chauhan, D.K., Parmar, N. and Verma, V. (2012). Role of genetic engineering in horticultural Crop improvement – A review.*Agri. Reviews*, 33 (3): 248-255.
26. Lucia, L., Costantini, E., Silvestroni, O., Pandolfini, T., Spena, A. and Mezzetti, B.

(2007). Auxin synthesis-encoding transgene enhances grape fecundity.*Plant Physiol-American Society of Plant Biologist*, 143(4): 1689-1694.

27. Silvia, J., Redondo, J., Blanco, J., Jose, L., Jose, M. and Aranda, L. (2002). Manipulation of strawberry fruit softening by antisense expression of a pectatelyase gene.*Plant Phy*siology, 128*:* 751-759.