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Prospects of transgenic fruit crops: A review paper

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Abstract
The major goal of plant scientists is to find ways to maintain high productivity under stress as well as developing crops with enhanced nutritional value in the era of increasing human population and climate change. Transgenic crops can prove to be powerful complements to those produced by conventional methods for meeting the worldwide demand for quality foods. Crops developed by transgenes can not only be used to enhance yields and nutritional quality but also for increased tolerance to various biotic and abiotic stresses. Although there have been some expressions of concern about biosafety and health hazards associated with genetically modified (GM) crops, there is no reason to hesitate in consuming genetically-engineered food crops that have been thoughtfully developed and carefully tested. In this review, we focus on the major prospects of transgenic fruit crops.

Keywords: transgenic, food, stress, crop, biotechnology

Introduction
Food insecurity and malnutrition are currently among the most serious concerns for human health, causing the loss of countless lives in developing countries. While the global area under transgenic crops continues to expand every year (James, 2011) [11], concerns have been expressed regarding unintended and unpredictable pleiotropic effects of these crops on human health and the environment (Dona, 2009) [6]. No harmful effects have been documented after several years of extensive cultivation of transgenic crops in diverse environments and consumption of genetically modified foods by more than a billion humans and by a larger number of animals. Also genetically modified crops can help us to meet the demand for high-yielding, nutritionally-balanced, biotic and abiotic stress tolerant crop varieties (Datta, 2013) [5]. Integration of modern biotechnology, with conventional agricultural practices in a sustainable manner, can fulfill the goal of attaining food security for present as well as future generations. There are many fruit crops like papaya, banana, mango, apple, plum, citrus, strawberry etc. has greater scope in production and nutrition as a transgenic

Review of Research Work
Papaya (B.N.: *Carica Papaya*, Family: Caricaceae)
Gonsalves *et al.* (1998) [8] conducted an experiment on comparative infection of transgenic and non-transgenic papaya in the 1995 field trial in Kapoho and found that all non-transgenic plants became infected within 11 months of starting the field trail while none of the transgenic test plants became infected. Ferreira *et al.* (2002) [7] conducted an experiment on comparison of bimonthly fruit yield of the hemizygous transgenic Rainbow cultivar of papaya and the Sunrise cultivar from the surrounding susceptible matrix 17 to 28 months after planting and found that Rainbow yields were over 2,242 kg/ha annually. For the susceptible Sunrise matrix plants, annualized yields were initially 419 kg of fruit per hectare and declined to less than 56 kg/ha after 1.5 years of harvest. Sakuanrungsirikul *et al.* (2005) [24] studied PRSV resistance, fruit production, and CP gene reaction (by PCR) of R3 (set 1) transgenic papaya under field conditions in Thailand and noticed in selection set 1, three R3 lines initially derived from transgenic papaya showed excellent resistance to PRSV (97%) and had a yield 70 times higher than non-transgenic papaya.

Plum (B.N.: *Prunus domestica*, Family: Rosaceae)
Scorza *et al.*, (1994) [24] and Ravelonandro *et al.*, (1997) [20] found transgenic plums (*Prunus domestica*) containing the PPV-CP transgene insert demonstrated various levels of resistance with the highest level shown by clone C5 (today named 'HoneySweet') which contained a multicopy insert and produced low levels of PPV-CP mRNA and no detectable PPV-CP. Capote *et al.* (2005) [2] compared the environmental impact that the release of transgenic plums to the field has on the dynamic and diversity of virus population.

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No differences in the serological or molecular variability of the PPV populations present in transgenic and non-transgenic plums was detected. Zagrai *et al.* (2011) [33] In their report showed that transgenic plum-trees expressing a PPV CP gene do not assist the emergence of PPV recombinants in the field under continental European conditions. The transgenic plums that are PPV susceptible and produce PPV-CP mRNA (except for the C-6 clone) were grown for 6-8 years in experimental orchards, and no significant differences in serological and genetic variability of PPV isolates sampled in transgenic plums and surrounding conventional plum trees was detected. Ravelonandro *et al.* (2013) [21] carried out pomological evaluation of external characteristics of transgenic plum 'Honey Sweet' and conventional plums and noted highest fruit balance (7), appearance (7), shape, weight (69.10), length (54.00), width (44.00), thickness (39.70), shape (3), colour (7) and flesh thickness (15.00). Furthur he carried out Plum fruit compositional analysis of transgenic and non-transgenic plums and the analyses showed that "Honey Sweet" (transgenic) fruit composition is generally in the range of the other plum cultivars tested.

Pear (B.N.: *Pyrus communis*, Family: Rosaceae)
Malnoy *et al.* (2004) [16] conducted an experiment on Fire blight susceptibility of in vitro transgenic clones (9A–10Q) compared to non-transgenic 'Passe Crassane' (PC). Fifteen days after inoculation, 80% of the non-transgenic 'Passe Crassane' plants had the highest rating of necrosis, while less than 3% of the 'OH' shoots (resistant control) were infected with low severity. Three clones (9X, 9M, 10M) showed a significant diminution of fire blight susceptibility compared to the non-transgenic control.

Pineapple (B.N.: *Ananas comosus*, Family: Bromeliaceae)
Sripaoraya *et al.* (2001) [27] transformed commercially important Thai pineapple (Ananas comosus `Phuket') were produced following microprojectile-mediated method for herbicide tolerance, into leaves of micropropagated shoots. Plants sprayed with Basta containing concentrations of glufosinate ammonium up to 1400 mg l^{-1} remained healthy and retained their pigmentation. They concluded that generation of herbicide-tolerant pineapple will facilitate more efficient weed control in this widely cultivated tropical crop. Trusov *et al.* (2006) [28] investigated the Silencing of ACC synthase gene *ACACS2* causes delayed flowering in transgenic pineapple and found that the delay calculated as the difference between the average flowering dates for the silenced transgenic plants versus TC controls was 69.4 and 52.1 days for line 1 and line 2, respectively, and was statistically significant.

Apple (B.N.: *Malus X Domestica*, Family: Rosaceae)
Murata *et al.* (2000) [17] conducted an experiment on PPO activities of transgenic and non-transgenic apple calli estimated and found that the PPO activity (0.25 ± 0.05) and browning potential (0.022 ± 0.006) in line As-a (transgenic calli) was about 50% of that in non-transgenic callus. Also the amount of expressed PPO protein in line As-a was about 50% of that in non-transgenic callus. Liu *et al.* (2001) [15] studied *in vitro* evaluation of shoots for resistance to E. amylovora in transgenic and non-transgenic line and found transgenic lines TR-1, TR-4, and TR-7 exhibited significantly greater levels of resistance. TR-5,TR-2 and TR-6 did not exhibit levels of resistance greater than that shown by control. While transgenic line TR-3 failed to exhibit a resistance level

different from that of non-transformed RG while the tetraploid (TR-3T) line was significantly more resistant the diploid shoot from which it is derived. Dandekar *et al.* (2004) [4] produced transgenic apple modified to synthesize endogenous ethylene. Ethylene suppressed fruits were significantly firmer than controls and found an increased shelf life, but no significant difference was observed in sugar or acid accumulation which suggest that sugar and acid composition were not altered. Ruhmann *et al.* (2006) [23] also showed that expression of a *stilbene synthase* gene does not affect the leaf shape, flower morphology or color, or fruit shape and size compared to control plants and fruit of *Malus x domestica* (cv. Elstar' and 'Holsteiner Cox). Wysocka *et al.* (2010) [32] investigated the stability of expression and the effect of the lytic protein gene, *attacin E*, on resistance to the fire blight disease in the apple cultivar 'Galaxy' grown in the field for 12 years. Using Southern and western blot analysis, they observed stability of expression of this gene in the leaves and fruit in several transformed lines. They did not detect any silenced plant. They concluded the expression of this gene increases in resistance to fire blight and did not affect fruit shape, size, acidity, firmness, weight or sugar level, tree morphology, leaf shape or flower morphology or color compared to the control. Weigl *et al.* (2015) [30] found the new approach of rapid cycle breeding in apple for the rapid introgression of important traits (e.g. disease resistances) from wild apple species into domestic apple cultivars (*Malus X domestica* Borkh.). This technique drastically shortens the long-lasting juvenile phase of apple. The utilization of early-flowering apple lines overexpressing the *BpMADS4* gene of the European silver birch (*Betula pendula* Roth.) in hybridization resulted in one breeding cycle per year. Wisniewski *et al.* (2015) [31] Carried out phenological disparities between non-transformed M.26 and Line T166 and found the difference in the time of bud break and the onset of leaf senescence was very prominent between the two lines, being offset by approximately 2 weeks. Induction of *MdEBB1* began earlier in the non-transformed M.26 trees, as did the occurrence of bud break. In contrast, expression in buds of T166 trees was induced about 2 weeks later and rose to higher relative levels. Artlip *et al.* (2014) [1] assessed Graft transmissibility of enhanced freezing tolerance from the transgenic T166 rootstock to the 'Royal Gala' scion. The LT_{50} in leaves of RG/M.26 versus RG/T166 were compared. Results indicated that there were no significant differences in the LT_{50} of leaves between the two types of trees. In contrast, leaves obtained from own-rooted T166 trees displayed a significant enhancement in freezing tolerance of −4 ^{0}C.

Strawberry (B.N.: *Fragaria vesca*, Family: Rosaceae)
Jimenez-Bermudez *et al.* (2002) [12] conducted an experiment on external and internal firmness in controls and transgenic Apel plants. 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, ranging the increment from 149% to a 179% when compared with macropropagated plants. Houde *et al.* (2004) [9] The WCOR410 protein was expressed in transgenic strawberry at a level comparable with that in cold-acclimated wheat. Freezing tests showed that cold-acclimated transgenic strawberry leaves had a 5 ^{0}C improvement of FT over wild-type or transformed leaves not expressing the *WCOR410* protein. However, no difference in FT was found between the different non acclimated plants. They suggested that the

WCOR410 protein needs to be activated by another factor induced during cold acclimation. There data demonstrated that the *WCOR410* protein prevents membrane injury and greatly improves FT in leaves of transgenic strawberry against freezing condition. Khammuang *et al*. (2005) [13] conducted PCR analysis revealed that all seven strawberry plants, transformed with the pSW1 gave band coresponseding to antifreeze protein gene and all three strawberry plants, transformed with pBB, also produced a band corresponding to the antifreeze protein gene. There were no bands of the AFP gene size appearing in control, non-transformed strawberry plants.

Mango (B.N.: *Mangifera indica*, Family: Anacardiaceae)
Chavarri *et al*. (2010) [3] studied PCR analysis of *Mangifera indica* L. transformed somatic embryos by wild *A. rhizogenes* (lanes 3, 4, 5 and 6) and non-transformed (lane 2) by the insertion of *Agrobacterium rhizogenes rolB* gene in Mango. In the agarose etidium bromide gels, the expected band (720bp) corresponding to the amplification of *rol B* gene, was observed in the transformed embryos, while no band was formed from non-transformed embryos.

Walnut (B.N.: *Juglans regia*, Family: Juglandaceae)
Walawage *et al*. (2013) [29] studied inhibition of root lesion nematode in roots of co-transformed somatic embryo genotype J1 and RR4 and found Lines 33-3-1 and 73-2-1 supported ~32% and ~36% fewer nematodes than the control nematode population, respectively. The least effective line, 66-1-2, still had ~10% fewer nematodes than the control nematode population. Out of the five single vector transformed lines tested, three lines (29-3-1, 2-1-1, and 15-3-1) controlled the nematode population completely (100% nematode reduction).

Banana (B.N.: *Musa paradisiaca*, Family: Musaceae)
Kumar *et al*. (2006) [14] studied the expression of Hepatitis B surface antigen in transgenic banana plants and found maximum expression level of 0.7 $\mu g\ g^{-1}$ (F.W of leaf) of HBsAg in pEFE HBs transformed plants grown in vitro. Namuddu *et al*. (2013) [18] The *Carica papaya* cystatin gene (*CpCYS-Mut89*) previously modified to improve its inhibitory potential against banana pests was introduced into the Sukali Ndiizi (ABB) cultivar using *Agrobacterium tumefaciens*, strain LBA4404 and the *gus* reporter gene was used to observe successful transformation process. Out of 28 selected lines, 27 were positive for both *hpt* and *CpCYS-Mut89* transgenes giving 96.4% transformation efficiency.

Citrus (B.N.: *Citrus sinensis*, Family: Rutaceae)
Soler *et al*. (2012) [26] has evolved three silencing suppressor proteins acting at intra- (p23 and p20) and ∕ or intercellular level (p20 and p25) to overcome host antiviral defence against Citrus tristeza virus (CTV). In summary, they provide the data showing that it is possible to achieve full resistance to CTV under controlled experimental conditions in a citrus host highly sensitive to the virus by RNAi targeting simultaneously the three viral silencing suppressors. While the complete sequences of the three genes were engineered in the construct to enhance its virus silencing efficiency, their start codons were mutated to make transgenes untranslatable in case of recombination with viral RNA.

Conclusion
So it can be concluded that there is a possibility of transforming fruit crop for a desired trait. Single insertion of CP genes of mild PRSV isolate give PRSV resistance capacity in papaya plant. Transgenic plum along with virus resistance shows the high quality fruit as well as nutritional value and hence reducing the environmental risk. Constitutive expression of an *EPS-depolymerase* transgene in pear decreases the susceptibility to fire blight by degradation of a significant amount of the EPS forming the bacterial capsules. Silencing of ACC synthase gene *ACACS2* causes delayed flowering in pineapple. Antisense method is useful for regulating the expression of PPO in transgenic apple. Overall, these results suggest that transgene expression in perennial species, such as fruit trees, remains stable in time and space, over extended periods and in different organs. This report shows that it is possible to improve a desirable trait in apple, such as the resistance to a pathogen, through genetic engineering, without adverse alteration of fruit characteristics and tree shape. The antifreeze protein from artic fish gives a major possibilities for the resistance against frost in crops. Induction of *MdEBB1* provides a better possibilities to delay bud break, shorten the plant height and girth in apple. In citrus it is possible to achieve full resistance to CTV by RNAi targeting simultaneously the three viral silencing suppressors. The study of *rol B* gene for mango transformation can play a major role in changing the desired traits. Also there is a possibility of expression of surface antigen of virus against hepatitis B in banana. There are many ethical issues related to the growing and consumption of transgenic crops. While the global area under GM crops continues to expand every year, no harmful effects of these crops have been documented even after several years of extensive cultivation in diverse environments and widespread human consumption (Ronald, 2011 and Park, 2011) [19, 22]. They hold potential to greatly increase the nutritional value of food as well as the productivity of crops, while at the same time provide many safety as well environmental concerns.

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