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The impacts of the transgenes on the modified crops, non-target soil and terrestrial organisms

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Agriculture forms a basis for survival and advancement of civilization of mankind and economic growth. The production and quality of food depends on nature of cultivars that have hitherto been obtained by mutation and hybridization of selected plants. Since the productivity of many crops has reached a plateau the gene transfer technology is used to break the yield barriers to meet the demand of the growing population of mankind. The gene transfer although yielded intended effects including reduction of emission of greenhouse gases, caused several changes in the modified crop plants, interacted with soil food webs, affected the biota associated with agro system, inflicted damage to mammals and birds, and contaminated both the related and unrelated plant species through horizontal gene transfer. The gene transfer also caused the phenomena of resistance and ecological succession of biota in different habitats

Key words: Gene transfer, transgene, agriculture, modified crops.

INTRODUCTION

Agriculture has been playing a key role in the development of human civilization from Pleistocene era (Peter et al., 2001). The biota that were present at the end of this era were similar to modern ones, brought in selective domestication and cultivation of plants and ensured the food and nutrient security to human population. As this selective method failed to meet the demand of growing population of mankind, cross pollination was resorted to by following the principles of plant genetics established by Gregor Mendel (Druery and Bateson, 1901) in the 19th century and inducted the desired traits into plants. However, the available genetic variability in the gene pool of wild species and the other cultivars had not met the goal for increasing the productivity of the crops. The mutation breeding with the chemicals or radiation has been employed for infusing the desired traits in the crop plants (Muller, 1930). Since these methods had not supported the human population the sexual hybridization was resorted to get the desired traits from different plants into one plant through cross-pollination and improved the productivity. The breeding techniques have reached its peak with the introduction of the dwarf gene into wheat (Borlaug, 1968) and rice (Qin et al., 2008). The dwarf genes and hybrids besides making them highly fertilizers

and pesticides responsive, removed poly crop system and laid the foundation for green revolution to prevent the starvation death of billions of people around the world. The uses of monoculture, pesticides and fertilizers have resulted in the degradation of environment, fertility of soils, nutrient status of the crops and health of living beings (Byrne, 1990; Menzie, 1972; Mozafa, 1993; Sarkar, 1990).

The discovery of DNA's double helix structure by Crick and Watson (1953) held the key to cracking the genetic code which determined how all living things work and this has formed the basis for the modern plant breeding techniques. The modern plant breeding is targeted to identify gene fragment of DNA for a particular trait in any species of plant, animal or microorganism, then isolated, copied, and introduced into an existing crop variety with the aid of *Agrobacterium* (Joos et al., 1983) or gene gun (Gan et al., 2000) or electroporation (Joshi and Schoenbach, 2000). This modern gene splicing method provided a more precise approach to crop improvement than sexual hybridization and was first developed in the 1983 (Estrella et al., 1983). A large number of crop species were genetically modified with the addition of any one of the traits for high productivity, nutrients, (Babili

et al., 2002), abiotic and biotic stress resistance (Vaeck et al., 1987), to reduce the impacts of global warming by capturing the carbon from the atmosphere (Jansson et al. 2010), pharmacological products for molecular farming (Chong and Langridge, 2000), for removal of pollutants from the environment by phytoremediation (Ma, 2010, Pascal et al., 2010,) and also to enhance the production of biofuels in micro algae (Beer et al., 2009). Many millions of hectares of commercially produced transgenic crops have been grown annually in a number of countries and has the greatest impact on global agriculture by way of increasing the farm economy to the extent of \$27 billion, reducing the use of pesticides by 172 million kg, reducing the release of greenhouse gas emissions from agriculture (Brookes and Barfoot, 2000) and water inputs on pesticide application (FAO, 2004). But the genetically manipulated plants do have some unintended effects as other methods (Davis et al., 2004) on the environment, non-target organisms, invite the phenomenon of ecological succession, causing biochemical lesions and interact with the health of living beings despite careful selection of crops.

EFFECTS ON THE SOIL ORGANISMS

It has been well established that the genetically manipulated plants affected the soil ecosystem. The genetically manipulated crop species by shedding the plant parts and sending the assimilates through roots as exudates deleteriously affected the useful soil microbes that are involved in the decomposition of the organic matter (Whipps, 1990). Most of the GM crops carry the Cauliflower Mosaic Virus (CaMV) 35S promoter which remains stable for long periods and caused several environmental hazards (Ho et al., 1999). The GM potato lines producing lectins GNA and ConA had altered the composition of starch and affected the microbial activity in soil (Griffiths et al., 2000; Milling et al., 2004). The modified alfalfa to produce lignin peroxidase significantly altered the composition and activity of rhizospheric bacterial communities (Donegan et al., 1999). The *Bacillus thuringiensis* (Bt) plant residues and root exudates lead to an accumulation of the transgenic gene (Dunfield et al. 2009) and protein in the soil (Saxena et al., 1999), persisted long (Tapp and Stotzky, 1998) as that of an insecticide DDT (dichlorodiphenyltrichloroethane) (Edwards, 1976) and lowered the microbial activity representing a potential risk for both target and non-target soil organisms. The Bt soybean and rice did not have any effect on the soil microorganisms (Ferreira et al., 2003; Wu et al., 2006). But the soil amended with Bt rice straw did lower the dehydrogenase activity, methanogenesis, production of hydrogen and anaerobic respiration (Wu et al., 2004). These changes might be due to the increased content of the saturated lipids with a considerable loss of unsaturated lipids from the soils (Dinel et al., 2003). However, a

dramatic change in the micro flora of the root surface was discerned in rice (Liu et al., 2004) that was modified for production of chitinases and glucanases against pathogenic fungi suggesting that the differential effects of genes employed. Some studies had also showed no apparent deleterious effect of the CryI Ab toxin released by Bt corn plants through root exudates or directly incorporated toxin into soil on earthworms, nematodes, protozoa, culturable bacteria, saprophytic fungi, Collembola and Acaridida (Saxena and Stotzky, 2001). The Gamasida, Oribatida and mycorrhizal fungi in microcosms were not affected by growing Bt-maize (deVaulleury et al., 2007). The glyphosate- and glufosinate-resistant *Brassica napus* and corn did not induce permanent alterations in the composition and diversity of soil and rhizospheric microbial communities (King et al., 2001; Dunfield and Germida, 2003). The abundance of bacteria and fungi was reduced by 23% in soils grown with potato expressing the cysteine proteinase as potato-cyst nematode (Cowgill et al., 2002). The incidence of pathogenic *Fusarium* fungi was increased by two-fold in glyphosate-resistant soybeans (Larson RL et al. 2006) due to varying soil moisture conditions and altered the root biomass, root nodules and the total nitrogen when sprayed with glyphosate (Means and Kremer, 2007), whereas the glyphosate- and glufosinate-resistant *Brassica napus* and corn did not induce permanent alterations in the composition and diversity of soil and rhizospheric microbial communities (Siciliano and Germida, 1999; Giovannetti 2005). The genetically modified (GM) brinjal expressing the defensin Dm-AMP1 had inhibited the growth of both phytopathogenic fungi and by releasing high levels of the antimicrobial protein in root exudates (Turrini et al., 2004).

The plant residues of the GM crops incorporated into soil was decomposed at a slower rate in the cases of Bt corn, canola, cotton, potato, rice and tobacco as compared to their non-Bt lines (Flores et al., 2005) due to the presence of a higher lignin content in the tissues of the plants (Saxena et al., 1999). The delayed decomposition might also be due to differential enrichment of bacterial population which was able to synthesize enzymes for degradation of complex carbohydrates in the Bt crop residues ploughed *in situ*. The lower rate of degradation of litter might be beneficial for the maintenance of soil structure. But longer persistence of Bt residues might enhance the risks related to toxin accumulation. The germinability and growth of presymbiotic hyphae of *Arbuscular mycorrhizal* fungus *Gomus mosseae* that converts the litter into humus, enhanced disease resistance, improved water relations, and increased soil aggregation in soils incorporated with Bt crop residues (Castaldini et al., 2005) and these changes perhaps due to differential rate of release of gene products from the modified crop litter. But the modified tobacco plants for the expression resistance had delayed root colonization by *Gomus mosseae* fungi (Vierheilig et



Figure 1. Heavy infestation of the Mealy bugs *Phenacoccus solenopsis* (Tinsley) in the cotton system due to ecological succession of insect pests in India.

al., 1993). The activity of soil microflora, dehydrogenase enzymes and respiration of soil and mineralization of nitrogen were reduced while the level of phosphorous was enhanced in soil grown with the Bt cotton (Sarkar et al., 2009). The root nodule bacteria with Bt transgene. Heavy infestation of the Mealy bugs *Phenacoccus solenopsis* (Tinsley) in the cotton system (Figure 1) due to ecological succession of insect pests in India displaced non-manipulated bacteria in legume root nodules (Skøt et al., 1994) and this may pose serious problems related to the fertility of soils. But the nitrogenfixing Diazotroph bacterial community did not suffer as evidenced in the Bt white spruce plantation (Lamarche and Hamelinthe, 2007).

EFFECTS ON THE MODIFIED PLANTS

The transgenes by interacting with the native ones caused several biochemical changes in the plants and altered morphological traits of the modified plants. The alien genes CP4 EPSP (Gertz et al., 1999), lectin gene (Bernal et al., 2003), phytoene synthase gene (Fray et al., 1995) and Bt gene (Shu et al., 2002) had induced dwarfism in soybean, sugarcane, tomatoes and rice. The GM soybean plants resistant to herbicide were with less chlorophyll and increased susceptibility to stem-splitting at high temperatures (Gertz et al., 1999). The Bt rice was inferior to non-transformed one in terms of plant height,

number of productive tillers per plant, number of grains per panicle and seed setting rate and produced smaller or malformed grains (Shu et al., 2002). The gene MdPG1 in apples had changed the composition of cell walls containing less pectin with malformed stomata which remained open during night (Atkinson et al., 2002) to cause higher transpiration resulting in utilization of more water than the normal trees. The Dandelions manipulated to have compound leaves had irregular leaf form and did not flower (Müller et al., 2006). The GM *Arabidopsis* plants producing biodegradable polyester were smaller, showed severe changes in metabolism and never produced seeds (Bohmert et al., 2000). The transgenic Lc alfalfa stem and leaves were bright green, synthesized more anthocyanins, lignin and proanthocyanidins with less flavone content and changed the quality of forage (Ray et al., 2003; Shadle et al., 2007). The genes *psy* and *beta-ly* were able to synthesize both β -carotene and xanthophylls in rice (Beyer et al., 2002). The altered biochemical make-up was evidenced in the transgenic pine apple and tobacco (Yabor et al., 2006; Misawa et al., 1994). The DHS gene in *Arabidopsis* delayed bolting and leaf senescence with bigger rosette leaves, root biomass, catalyzed the conversion of lysine to deoxyhypusine and enhanced yield (Wang et al., 2003). The phytoene synthase gene delayed the germination and increased levels of chlorophyll and carotenoids (Lindgren et al., 2003). The heat-stable enzyme beta-glucanase gene had lowered the grain weight and yield of barely (Horvath et

al, 2001). The barley with zinc transport protein gene had negative effect on the uptake of zinc in zinc-deficient soil (Ramesh et al., 2004). In some cases, the transgenes yielded non-viable off springs as in barley (Bregitzer et al, 2007). The transgenic birch trees expressing an anti-fungal enzyme chitinases showed increased susceptibility to leaf spot disease in the field (Pappinen et al., 2002). The delayed germination, reduced the level of chlorophyll with changed structure of plastids and composition of fatty acids, and decreased the level of gamma tocopherol were evidenced in GM Canola (Shewmaker et al., 1999). The tissue that had low chlorophyll content did not express fully Cry1A in maize and cotton (Abel and Adamczyk, 2004). The silencing of gene occurred in Bt cotton at 37°C during bolling stage and altered the levels of toxin, glutamic-pyruvic transaminase, soluble protein, increased levels of protease and free amino acids (Chen et al., 2005). Glyphosate-resistant cotton had produced 40% less loose pollen per stigma with a variety of cellular abnormalities and increased the distance between the anthers and the stigma (Pline et al., 2002). The stems of Bt maize plants had significantly lower levels of alkenes. But they were richer in n-alkanes, n-fatty and unsaturated fatty acids than non-Bt shoots to affect the digestibility in cattle or pigs (Poerschmann et al., 2008). The silking and maturity were delayed with the lower yield, and higher moisture content of grain in Bt maize than in the conventional varieties suggesting that the modified plants drained much of water from the system and yielded less (Ma et al., 2005). The chitinase gene in pineapple had decreased the levels of aldehydes, chlorophyll a, b, phenolics and proteins and thereby reduced ability to perform photosynthesis and withstand stress tolerance (Yabor et al., 2006). The alien gene in sugarcane had modified the sugar metabolism (Vickers et al., 2005a). The sense or antisense constructs engineered had significantly lowered the cane yields with darker juice and higher levels of polyphenol oxidase derived from a sugarcane cDNA library (Vickers et al., 2005b). The glucokinase gene in potato had significantly decreased the amount of starch in the tubers by accumulating the hexose phosphates, organic acids and amino acids and lowered the levels of both alpha-chaconine and solanine glycoalkaloids in the leaves and also caused changes in 88 metabolites including alkaloid content of the plant as evidenced in potatoes (Trethewey et al., 1998; Birch et al., 2002; Roessner et al., 2001a). The blast resistance gene (Coca et al., 2006) in rice had activated the oxidative-stress response for enhancing the ceropin A protein synthesis and folding for subsequent secretion (Campo et al., 2008). The genes for the HIV proteins p24 had slowed down the growth of tobacco plants due to yellow pigmentation and induced rearrangement of DNA (Zhang et al., 2002). It was reported that the modified barley for full-length collagen protein of human was found at low and detectable levels in its seeds. But the next generation had fertility problems and produced seeds with no

detectable human collagen (Eskelin et al., 2009). The flavonol-enriched tomatoes with LC and C1 genes had altered levels of at least fifteen substances including citric acid, sucrose, phenylalanine and trigonelline (Le Gall et al., 2003). The antisense DHS gene in tomatoes by increasing the content of chlorophyll enhanced photosynthesis which enabled the plant to have increased starch. But the pollens in these plants were deformed and necessitated cross-pollination for formation of fruits (Wang et al., 2005). The transformed wheat with a novel "hybrid" glutenin subunit (HMW-GS) gene showed an irregular expression of glutenin and changed its expression levels over subsequent generations (Blechl and Anderson, 1996) while the low-molecular-weight glutenin subunit gene had increased the expression of glutenin and decreased the gliadin synthesis in wheat (Masci et al., 2003). The wheat with scab-resistance genes caused localized death of leaf tissue due to formation of necrotic spots on the leaves during the flower formation stage of the crop (Anand et al., 2003). Interestingly, the single-site integration of foreign DNA into *Arabidopsis* had undergone rearrangements and deleted both plant DNA as well as foreign DNA (Forsbach et al., 2003). The anti-sense shikimate hydroxycinnamoyl transferase gene with PAL2 promoter in alfalfa caused stunting with less biomass and delayed flowering and reduced the enzyme activity and lignin content to improve the digestibility of forage (Shadle et al., 2007). The flax seeds expressing the bacterial phytoene synthase gene crtB, showed elevated level of carotenoid and changed its components, i.e., α -carotene, 13-carotene, phytoene and lutein (Fujisawa et al., 2008). The carotenoids are considered as a strong protective agent against lung cancer (Nishino et al., 1995) and lutein is an essential functional component in the human eye (Berendschot et al., 2000). In canola or *Arabidopsis* or tomato, the phytoene synthase genes *scr113* or *FSY* respectively, resulted in delayed germination (Lindgron et al., 2003) produced darker seeds, increased the level of α -carotene, lutein, violaxanthin, zeaxanthin, lycopene and chlorophyll (Shewmaker et al., 1999). There was over accumulation of starch in leaves of potato, inhibition of growth, photosynthesis, induced rapid senescence, decreased fruit setting, sucrose unloading capacity and delayed softening of fruit in tomato that were transformed with the antisense genes (Veramendi et al., 1999; Dai et al., 1999). Also, in tobacco, the phytoene synthase gene resulted in severe phenotypic changes, altered the morphology and pigmentation of leaf (Busch et al., 2002). The Bar transgenic oats exhibited greater growth and increased tolerance to salt stress conditions as against no GM crop (Oraby et al., 2005) in which both the transgenic construct and host DNA got modified (Wojciede et al., 1998) and this phenomenon may have negative consequences on the biodiversity. However the organ specific gene promoter like LP2 was claimed to limit the potential unintended impacts on plants and environment (Thilmony et al., 2009) and such promoter will go in a long

way to help in generating the new crop varieties that may not differ from that of their isogenic lines. The plant volatiles have profound effects on the composition and visitation of insects community to the cropped area. The transgenic scab resistant apple differed quantitatively in four terpenes and an aromatic compound and these modified odour emissions proved to be crucial in the composition of non target insects (Ute et al., 2010).

EFFECTS ON THE NON-TARGET ORGANISMS

The pollen is a potential source for exposure to non-target insects and consumption of deposited pollens on plants affected the non-target insects as well as pests (Felke et al., 2002). The survival of monarch butterfly *Danaus plexippus* larvae to adulthood was reduced when they were exposed to Bt maize pollens (Dively et al., 2004). But simultaneous exposure to both Bt anthers and pollens caused an additive effect and resulted in a lower survival of monarch larvae (Anderson et al., 2005). A similar effect was also noticed in *Propylea japonica* fed with the rice pollen (Bai et al., 2005). The predators and parasites of insect pests of crops were also succumbed to the genetically modified crops (Ahmad et al., 2006). The longevity of the predators *Orius tristicolor* and *Geocoris punctipes* that were raised on the prey fed on Bt cotton was significantly decreased by 27 - 28% whereas *Nabis sp.* and *Zelus renardii* remained unaffected (Ponsard et al., 2002) and differential susceptibility might either be due differential quantity of food consumed or elimination of the toxin from the system. The hymenopteran parasitoids often showed adverse effects while parasitizing host fed on Bt plants due to the poor quality of the host (Lövei et al., 2005). The Cry1Ac in cotton retarded the development of hymenopteran parasites that were raised on their hosts possibly due to sub-lethal effects on the host (Baur and Boethel, 2003). Similarly, an important endoparasitoid of the cotton bollworm *Microplitis mediator* in China suffered with reduced survival and growth inhibition when its prey *Helicoverpa armigera* raised on Bt cotton leaf powder (Cry1Ac) (Liu et al., 2005). The survival, developmental period and weight of cocoon were negatively affected in *Cotesia marginiventris*, *Aphidius* (Vojtech et al., 2005; Ashouri et al., 2001), *Parallorhogas pyralophagus* (Tomov et al., 2003) on hosts that were fed on the hosts fed with the modified crops with Cry1Ab, Cry3A and Cry9C. The abundance of some hymenopteran parasitoids was lowered in fields cropped with Bt maize and Bt cotton as compared to Bt corn (Cry1Ab), where the abundance was increased (Dively, 2005; Pilcher et al., 2005). Bt rice did not either resurged the populations of plant hopper or negatively affected its predator *Cyrtorhinus lividipennis* (Chen et al., 2007).

The predator larvae of *Chrysoperla carnea* had shown a preference for prey fed on the non- transgenic corn as

compared to prey fed on Bt corn (Meier and Hilbeck 2001) which would potentially reduce the exposure of this predator. The tritrophic interaction study revealed that the pupal body mass of lacewing *Chrysopa pallen* fed with Bt cotton GK12-originated aphid prey was significantly higher than that of the control and more number of females were emerged. These females were laid significantly more number of eggs than the females emerging from larvae maintained on another Bt cotton variety, NuCOTN 99B suggesting that the differing nutrient status of the hosts (Guo et al., 2008) might be responsible for this effects. The risks for predators, Lacewings *Chrysoperla carnea* when feeding on thrips, *Frankliniella tenuicornis* in or next to Bt maize fields were found to be negligible (Obrist et al., 2005) and a similar effect was noticed in predatory ladybeetle, *Propylaea japonica* on cotton aphids (Zhu et al., 2006).

The insect predatory pirate bug, lady bird beetles, and carabids did not suffer under Bt corn (Daly and Buntin, 2005). A five-year field study showed that the Bt cotton had not altered the activity of the natural enemy community on three key pests but a field study made over three years had clearly shown that the population of ladybird beetle was lowered in Bt cotton fields due to availability of reduced number of prey (Naranjo, 2005b). However, the abundance was lower in pure Bt cotton plots as compared to a row mixture of Bt and non-Bt plants in another field study (Sisterson et al., 2004). The overall arthropod diversity and the diversity of pest sub-communities were increased, but the diversity of natural enemy sub-communities were decreased in Bt cotton field (Men et al., 2004) and the density shifts of natural enemies were often ascribed to prey dynamics or plant-mediated indirect causes. The egg plant expressing the Cry3Bb toxin were preferred by the herbivorous spider mites, *Tetranychus urticae*, but its predator, *Phytoseiulus persimilis* consumed significantly less number of Bt-fed spider mites (Zemkov et al., 2005). However, the jumping spiders and Lacewings, *Chrysoperla carnea* were found to be less abundant in Cry1Ab × Vip3A cotton (Dively 2005). But spiders as a whole were more numerous in Bt maize (Ahmad et al., 2006), cotton (Head et al., 2005) and potato (Reed et al., 2001). The Bt potatoes expressing Cry 3Aa had no effect on the aphid *Myzus persicae* and aphidophagous coccinellids *C. septempunctata* fed on the aphid (Kalushkov and Nedved, 2005). The recent study elucidated that the ecological function of natural predation on the eggs of *Leptinotarsa decemlineata* in GM potato plots was not impaired (Arpaia et al., 2009). It was evident that the direct environmental benefits of reduced insecticide applications in Bt-cotton resulted in fewer non-target effects (Whitehouse et al., 2005).

Although the pollen containing the Bt toxin is harmless to bees, their visitation to the fields cropped with modified Canola (van Engelsdorp et al., 2010; Morandin and Winston, 2005) resistant to herbicide was reduced and this phenomenon is considered as a threat to humanity

as predicted by Albert Einstein. The weed seed resources for birds were reduced in glyphosate-resistant beet production areas and this phenomenon poses a great threat to the bird community (Gibbons et al. 2006). Interestingly the woodlouse, *Porcellio scaber*, performed better when fed with Cry1Ab maize as compared to the non-transgenic isolate, which was attributed to a better nutritional quality of the Bt corn (Riudavets et al., 2006). The Bt rice grains had affected variously the stored product insects, *Plodia interpunctella*, *S. oryzae* adult and the psocid *Liposcelis bostrychophila* (Escher et al., 2000) suggesting that the Bt toxin is capable of getting into the food chain.

EFFECTS ON THE MAMMALS AND POULTRY

The genetically modified (GM) crop plants have allergens and caused different kinds of allergies both in the test animals and human beings. A new molecule (SSP 6711) expressed by GM maize was akin to 50 kDa gamma zein is a well known allergenic protein and also carried several synthetic RNA fragments to interact with the mammalian immune system to cause unexpected turbulences (Nordlee, 1996; Zolla et al., 2008; Rosati et al., 2008). A recent study in GM maize had identified the recurrent changes in the immune system involving changes in the number of a special type of lymphocytes and is important in the human (Rachmilewitz et al., 2004) as well as mice immune system (Finamore et al., 2008). The GM peas expressing non-native proteins in plants resulted in the structural variants possessing altered immunogenicity in mice (Prescott et al., 2005). The modified rice seeds with genes from Japanese cedar pollen allergens were shown to function as an effective edible vaccine for the control of pollen allergen-induced responses (Takagi et al., 2006). Interestingly the accumulation of allergen Gly m Bd 30 K (P34) in the seeds of Soya bean was prevented by modifying the plants with a silencing gene (Herman et al., 2003).

The GMO foods were affected the normal physiology and biochemical metabolisms in the mammals. The rats fed with GM maize had significant variations in growth in both the sexes and female suffered from hepatorenal toxicity with increased triglycerides, while the males excreted diminished amount of phosphorus and sodium in the urine (Séralini et al., 2007) and significant disturbances in the function of kidney, liver and some effects on heart, adrenal, spleen and blood cells were also frequently seen between male and female rats fed with GM maize (deVendômois, 2009). The haemorrhage, sickness, and lesions in liver with the altered enzyme production, enlarged pancreas, stomach with mysteriously heavier intestine and proliferation of the gastric mucosa on different parts of the gastrointestinal tract were noticed in rats fed with GM foods (Pusztai et al., 2003) rats fed with the potatoes engineered to produce a

lectin showed proliferative cell growth in both the stomach and intestinal walls (Stanley et al., 1999), partially atrophied livers and slower growth in the brain (Pusztai, 2002). The Cell proliferation was reported to be a precursor to cancer (Pusztai, 2005). The FlavrSavr GM tomato fed rats died within two weeks (Pusztai, 2002) and GM Soya food increased the infant mortality of rat by 55.3% (Ermakova, 2006). Rabbits fed with GM soybean showed higher metabolic activity with altered enzyme production in their livers (Tudisco et al., 2006). The cells in the pancreas of mice fed with the Roundup Ready soy had produced significantly less digestive enzymes (Malatesta et al., 2003). Embryos of GM Soy-fed mice also showed temporary changes in their DNA function (Vecchio et al., 2004). The microscopic analysis of the livers of mice fed with GM soybeans revealed altered gene expression and structural and functional changes. Enzyme production in the hearts of mice was altered by GM Soya (Tudisco et al., 2006). A high pup mortality was characteristic of every litter from mothers fed with the GM soy flour of R21 (Ermakova, 2006). Mice fed with the Bt potatoes showed an abnormal and damaged cells, and proliferative cell growth in ileum (Nagui et al., 1998). The ultrastructural analyses of the liver showed that the nuclei of cell of the GM Soya fed mice were more irregular in shape with a higher percentage of dense fibrillar components with lower proportions of fibrillar centres and granular component (Malatesta et al., 2002). The larger zymogen granule in pancreas with significantly lower α -amylase antibody labeling density and pancreatic α -amylase were noticed in animals fed with the GM soybean (Caporaloni et al., 2002). In both mice and rats fed with the Roundup Ready soybeans, dramatic changes in the testicles were observed; they were dark blue in colour instead of pink with the altered young sperm cells (Cisterna et al., 2006). The testis had higher number of perichromatin granules with dilated vesicles in cells and smooth endoplasmic reticulum of Sertoli cells. A temporary reduction in antibodies was evident and the female gave birth to fewer smaller babies than the normal one (Velimirov et al., 2008). Both the male and female animals displayed troubling reproductive problems when fed with the GM Soybean (Tudisco et al., 2006). The other study showed that the genetically modified crops had no adverse effects on the multigeneration reproductive-developmental ability (Rhee et al., 2005). The GM fruits of the brinjal modified with Cry1Ab and Cry1Ac sequences were known to have resistance to kanamycin, provided less calories and affected the intake of food as well as feed conversion rate in the test animals besides changing the picture of the blood cells and its chemistry in respect of the hepatic markers alanine albumin, lactosedehydrogenase and aspartate aminotransferases in animals. Interestingly, the milk production and its composition were changed by 10 to 14% in cows fed with the GM fruits (Seralini, 2009). The ileal starch, amino acids digestibility and apparent metabolisable energy (AME) were

significantly reduced in the birds fed with the transgenic up peas (Li et al., 2006). The Round up Soya carrying the stop signal had four harmful RNA variants which are considered as harmful and (Rang et al., 2005) reduced clinically important phytoestrogens levels by 12 to 14% (Lappé et al., 1999). Interestingly the hamsters fed with GM Soya showed the growth of hairs inside the pouches of the mouth and the number of hairy mouthed hamsters was much higher in the third generation of GM soy fed animals than in others (Baranov et al., 2010). The study with human showed that the antibiotic resistance marker gene survived in the gut of the ileostomy patients given one meal of GM Soya and detected the measurable amounts of the full length of the transgene construct (Susana et al., 2002). The full length transgene construct is likely to encode the functional gene provided if it is not methylated (Orend et al., 1995) and penetrate into the nucleus by crossing through the nuclear membrane (Gorlick et al., 1996). The large scale introduction of GM crops in USA had increased the incidence of three or more chronic disease of people from 7 to 13% (Paez et al., 2009).

THE HORIZONTAL GENE TRANSFER

The persistence and movement of transgenic DNA in agricultural and natural systems poses a threat of horizontal gene transfer and possible proliferation of genetically modified DNA into the general environment. Although the natural horizontal gene transfer (Ochiai et al., 1959) is limited by species barriers engineered artificial genetic constructs crossed all species barriers and invaded all genomes. There are many studies confirming that the pollens are contributing much towards the horizontal gene transfer in the plant kingdom (Jarosz et al., 2005). The pollens of GM maize are capable of moving up to 1000m and pollinate rape (Scheffler et al., 1993), sugarbeet (Alibert et al., 2005) and maize (Loos et al., 2003). The GM *Arabidopsis thaliana* with the Csr1-1 gene (Bergelson et al., 1998) and canola with bargene (Snow et al., 1999) were more freely pollinated the natural wild populations of *B. rapa* than the non GM plant. The F1 hybrids formed due to such trespass of genes competed with other weeds in the commercial agricultural fields (Warwick et al., 2003). The genes flow from GM rice (Chen et al., 2004) crossed into the population of creeping bent grass (*Agrostis stolonifera*) and *Zoysia* grass through pollens (Watrud et al., 2004). The low genes flow was also reported from GM rice, red rice and conventional rice (Messeguer et al., 2004), from transgenic rice to red rice (Zhang et al., 2003) and insect resistant rice (Bashir et al., 2004). The non-Bt plants in the refuges known to carry one to two copies of the Bt gene (Heuberger et al., 2008) and such contamination is detrimental to the system. The vector-mediated horizontal gene transfer and recombination could occur to create new pathogenic organisms

(Gebhard and Smalla, 1998) in the biosphere. A rare event of transference of transgenic gene was occurred from the plant to terrestrial bacteria (Nielsen et al., 1998). The persistence and movement of genes from GM crops through DNA pose a threat for possible proliferation in the general environment. The plant litters on decomposition released DNA spread into the soil rhizosphere, persisted for a long time upto two to several years and retained its transforming activity (Ceccherini et al. 2003, Gebhard and Smalla, 1999) in the soils towards naturally competent *Bacillus* (Blum et al., 1997), *Pseudomonas* (Meier and Wackernagel, 2003) and *Acinetobacter* (deVries et al., 2001) depending on homologous sequences in the recipient cells. Such DNA were protected by the clay minerals and other soil particles against degradation (Lorenz and Wackernagel, 1992). Although the DNA in decaying plant cells was rapidly degraded DNA of the appropriate length survived in some soils (Hay et al., 2002) and is prone for uptake. The plasmidic DNA detected in compost and manure was incorporated into soil (Smalla et al., 2001; Loos, 2003). It was known that the transgenic DNA concentration in bulk soil micro arthropods, nematodes, macro arthropods and earthworms was significantly higher than that of background soil (Miranda et al., 2009). The gene transfer between GM plants and fungi was detected both in co-culture systems and in planta (Bryngelsson et al., 1988) and could represent a potential risk in the case of fungal pathogens and symbionts living inside the host plant cells. Horizontal gene transfer was occurred from a GM potato line to a bacterial pathogen (Schluter et al., 1995), and the *in situ* transfer of gene occurred from GM plants to terrestrial bacteria (Kay et al. 2002) and also from GM zoysiagrass to the indigenous microorganisms in the cultivated field (Bae et al., 2008). The agro infection of transgenic plants had resulted in causing viable cauliflower mosaic virus disease through the processes of intermolecular recombination (Gal et al., 1992).

All though there were conflicting reports on the fate of GM DNA in the biological system it was observed that DNA could pass through the gut wall into the blood stream and taken up by cells in the blood, liver, spleen and passed through the placenta to the cells of the foetus and the newborn one (Doerfler and Schubert, 1998). The presence of recombinant DNA fragments and CryIAb protein was detected in the gastrointestinal contents of pigs (Chowdhury et al., 2003), mice (Schubert et al., 1997), oral cavity and rumen of sheep and the digestive tract (Duggan et al., 2004) that were fed with GM food. The gene from GM Soya crop was transferred into the intestinal bacteria and continued to function in the human system (Netherwood et al., 2004). The fragments of synthetic transgenes were also traced in the blood of piglets fed with GM maize food (Mazza et al., 2005). The transformation activity of DNA of decaying GM maize in ovine saliva, rumen fluid and silage effluent and found the way into aquatic environments was also occurred (Paul et al.,

1989; Duggan et al., 2000). The gills, digestive glands, and gonads of the fresh water Mussels were significantly contaminated by cry1 and cry1Ab genes (Douville et al., 2009). Interestingly the DNA and proteins of transgenic pollen were remained intact in honey for more than seven weeks (Eady et al., 1995). Interestingly the deleted gene npll was able to take up and integrate npll and other genetic material from transgenic sugar beet through homologous recombination (Gebhard and Smalla, 1998).

EFFECTS ON RESISTANCE AND ECOLOGICAL SUCCESSION

The ecological succession is a natural phenomenon when one species of competing organism is displaced from the habitat either by nature or anthropological activity (Sundaramurthy, 2002) will lead into the succession of another one as evidenced earlier in the Indian cotton system on the account of gene to gene interaction (Sundaramurthy, 1994). The large scale cultivation of genetically modified crops over several years could increase the selection pressure on pest species, which might result in the development of resistance (Fox, 2003). The resistant population may elicit succession within the species in the given habitat by breeding with the susceptible wild one. Bt cultivars showing large reductions in Cry1Ac concentration over the growing season became more susceptible to insect damage (Olsen et al., 2005). Increased resistance to Bt toxin was noticed in the populations of American bollworm at high temperatures as the high temperature altered the synthesis of the protein metabolism by silencing the gene by way of reducing the glutamic-pyruvic transaminase, soluble protein and increasing protease and free amino acid levels in the leaves (Chen et al., 2004). The insect species diamond-back moth (Martinez-Ramirez et al., 1995). European corn borer (Weinzierl et al., 1997) pink bollworm (Wilson et al., 1992) had developed resistance to the transgenic Bt crops and the resistant population would cause succession by breeding with the susceptible one in the ecological niches. The changes in the biochemical make up of the modified crops (Martinez-Ramirez et al., 1995) may accelerate succession of other insect pests of minor importance as noticed earlier with indiscriminate use of synthetic pyrethroids insecticides (Sundaramurthy, 1994) and currently widespread growing the transgenic cotton (Nagrare et al., 2009) in India and China (Lu et al., 2010). The genetically modified crops are also known to cause succession of pathogenic fungi due to availability of certain nutrients. The root exudates containing elevated levels of several amino acids, fructose and maltose in transgenic cotton supported the growth of the fungus, *Fusarium oxysporum* and resulted in succession (Li et al., 2009). However, GM beet cultivars showed significantly more injury from the soil fungus *Fusarium oxysporum* and *Rhizoctonia solani* when sprayed with glyphosate which could have sequential effect by increasing the inoculum

of soil pathogens and affect other crops in rotation with GM sugar beet (Pasonen et al., 2006). The transgenic birch trees expressing an antifungal enzyme gene had increased the susceptibility to leaf spot disease in the field (Pasonen et al., 2004). The significant biological disadvantage of transgenes is that they may either transform wild weed plants into new or worst weeds or cause ecological succession of existing weed species resistant to weedicides as in Argentina (Vitta et al., 2004).

The techniques involving the use of the restriction enzymes Zinc-finger nucleases for inducing a double-stranded break at their target locus (Shukla et al., 2009) and Targeting Induced Local Lesions in Genomes (TILLING) (Koornneef et al., 1982) that combines chemical mutagenesis with a sensitive mutation detection instrument are showing promise in the field of genetics for improvement of the crop plants (Reynolds et al., 2009) by accelerating the evolutionary process without involving introduction of foreign DNA of any type into a plant. The Cisgenesis (Giddings, 2006) involving a natural gene (Rommens et al., 2004) from the breeder's gene pool that codes for a trait also shows promise in generating the crops with high yield, enhanced nutritive values, resistance to biotic and abiotic stresses. This concept may facilitate second green revolution not only in the Indian agro system (Jacobsen et al., 2008) but also elsewhere for conferring food and nutrition security to mankind without causing any immediate disturbances to the available water resources, health of living beings and biodiversity.

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