Chapter 1
Transgenic Crops to Preserve Biodiversity

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Abstract The rapidly expanding field of commercial transgenic cultivation has its greatest concern related to environmental well being as transgenic crops are seen as a threat to the biodiversity in the agricultural fields. Since transgenic technology is continuing to witness a rapid growth in terms of developing novel varieties, it is imperative to examine whether the developed varieties contribute to preserving biodiversity. Further, it is also necessary to focus future research towards developing transgenic varieties to contribute to preserving and enhancing the biodiversity. The present review aims to present an overview of the current status of transgenic technology in contributing to biodiversity and suggest future research strategies enabling the preservation of biodiversity.

Keywords Crop biodiversity · Transgenic crops · Speciation · Non-target species

1.1 Introduction

Biodiversity as a term is a shortened version of 'biological diversity' where 'diversity' as a concept encompasses the range of variations or differences among entities that have a distinct and independent existence. Thus, biodiversity can refer to the number, variety and variability of living organisms (Groombridge and Jenkins 2002). A broader reference to biodiversity can be as a collective indicator of the numerous forms of life and of ecological habitats on planet earth (Convention of Biological Diversity 2000), where it is a crucial factor that affects both the survival and welfare of our existence as a species. To effectively manage and conduct both qualitative and quantitative research on the various facets of biodiversity, three fundamental hierarchies were delineated as: *genetic diversity* (representing the heritable variation between population of organisms in terms of their DNA base pairs and the genetic code); *species diversity* (representing the 12.5 million global species

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in different taxonomic groups of which 1.8 million species have been described to date) and *ecosystem diversity* (representing the abiotic components determined by soil parent material and climate) (Groombridge and Jenkins 2002).

Human intervention has been responsible for the continual degradation of biodiversity and this led to numerous economic, environmental, and social consequences. The failure to preserve our natural biological resources especially the diverse plant life on which we depend for food, clothing, pharmaceuticals, and more recently energy in the form of biofuels is indicative of a fact that we may be losing potentially beneficial compounds and materials that have not yet been discovered from natural resources (OECD 2005). More recently, research has shown that climate change is having a significant effect on the world agricultural output and thus directly influences world food security (White et al. 2004). For decades, the development of novel crops by both conventional breeding as well as biotechnological crop improvement strategies had a direct influence on world food security (Kropiwnicka 2005). Plant biotechnological strategies that focused on the development of improved high-yielding and disease resistant crop varieties were a result of collaborative efforts between conventional and molecular breeders (Van Buerren et al. 2010). A logical continuation of such research collaborations can now culminate towards focused research studies resulting in preserving and enhancing plant biodiversity.

Plant biotechnological research was witness to many path breaking and application-oriented technologies (Kumar et al. 2009) and it sometimes is a challenge for current researchers to identify contextual research strategies without getting lost in the diverse array of biotechnological strategies that unfold every year. In order to develop novel strategies to preserve plant biodiversity (Pijut et al. 2011), researchers will be best advised to focus on certain crucial aspects of plant molecular biology to better utilize the tools of biotechnology that are available.

1.2 Transgenic Crops

Transgenic crops are a result of the process of *transgenesis* (also referred to as plant genetic engineering) that involves the introduction of a desirable exogenous transgene in crops and ensuring their stable expression in the offspring. The process of transgenesis relies on stable integration, desired level of expression, and predictable inheritance of the introduced transgenes. In the context of introducing novel crop varieties and the strategies that enable introduction and expression of genes of interest across any crop species, transgenic technology is now being hailed as a gene revolution that is similar to the plant breeder-pioneered green revolution of the 1950s (Pingali and Raney 2005; Jain 2010). Green revolution owed its success to identifying strategies that effectively disseminated novel cultivation techniques and distributing the resulting improved germplasm freely to targeted farmer populations thus working towards greater public wellbeing (Jain 2010). This resulted in increased staple crop yields and a simultaneous price decrease that benefitted farmers in Asia, especially for crops such as rice and wheat among farming communities of Asia (Barta 2007). Comparison of the green revolution to the transgenic technology and christening it as a gene revolution has to overcome the purported empty rhetoric to a concerted identification of strategies similar to the success of green revolution related to the increased yields and the resulting economic benefits to farmers. Further, the major limitation attributed to green revolution in terms of increased monocultures that allegedly led to a decrease in biodiversity may also be a debatable issue. Transgenic research is also seen largely as a private enterprise with varieties available to farmers only on market terms (Pingali and Raney 2005) and this may jeopardize its cause to contribute to increasing biodiversity.

1.2.1 The Current Status of Transgenic Crop Cultivation

Transgenic crops (or the popular term attribute to them being genetically modified crops or GMOs) are increasingly becoming a common feature of cultivated landscapes with the total plantings seeing a significant increase from 3 million hectares in 1996 to 67.5 million hectares in 2003 (James 2003). Transgenic technology has also been showcased as having a positive impact on commercial farming (Carpenter 2010) and as an effective research alternative to meet to the global needs of food security (Schiøler and Pinstrup-Andersen 2009). The pioneering efforts as exemplified by the herbicide-tolerant soybean (Carpenter and Gianessi 1999), insect tolerant Bt-corn (Hilbeck 2001) and the more recent successes of the genetically engineered-vitamin A fortified “golden rice” (Beyer 2010) has led to the approval of a new generation of transgenic crops that produce health benefitting vitamins and vaccines, and economically important enzymes and industrial products (IUCN 2007). The rapidity with which over 16 million farmers of the global agricultural community has taken up the transgenic technology has surpassed all innovative agricultural practices of the past 80 centuries (James 2011; Lawson et al. 2009). Since the first commercial transgenic crops of China of 1992, the countries that rapidly adapted the transgenic crop cultivation were the USA followed by Argentina, Brazil, Canada and India (GM Compass 2009) with the total transgenic crop cultivation area registering an increase from 134 million hectares in 2009 to 160 million hectares by the end of 2011(James 2011). The four decade old global biotechnology industry has the United States leading the world in the rapidity of transgenic technology acceptance with proportions of major transgenic crops as high as 73 % in maize, 87 % in cotton and 91 % for soybean (USDA 2007/2010). In contrast, the opposition to transgenic technology has been so severe in the European Union with only 114, 500 ha that accounts for less than 0.01 % of European agriculture area mostly in Spain cultivates *Bt*-maize (James 2011).

1.2.2 The Advantages and Limitations of Transgenic Technology

The major advantages of transgenic technology as put forth by the proponents of biotechnology center around the careful and planned introduction of insect and herbicide resistance into arable land that would reduce the crop losses due to weeds, insect pests, bacterial and viral pathogens, thus providing an environmental-friendly agrochemical free atmosphere (Krimsky and Wrubel 1996) that will eventually contribute to a sustainable agricultural environment (Braun and Ammann 2003). This optimistic view is not shared universally across the plant biology scientific community, where certain researchers compare the embrace of transgenic technology without proper methods of risk assessment to the pesticide overuse of the twentieth century agriculture (Krebs et al. 1999; Herren 2003).

Herbicide resistant sugar beet were shown to receive lesser number of herbicide sprays in farm-scale evaluations (Champion et al. 2003), but the same was not observed in case of transgenic oilseed rape and maize. However, research in developing transgenic herbicide varieties has been seen as having a significant potential beneficial effect on environment and biodiversity as the reduction in pesticide use would reduce the pesticide-induced mortality of natural enemies, a key aspect of conservation biological control and integrated pest management (Barbosa 1998; Gurr et al. 2003).Transgenic herbicide resistant crops also aided in creation of precise patterns of weed strips connecting field margins with field interiors, beetle banks, and networks of habitat corridors favoring beneficial arthropods that enabled farmers to develop easier weed management (Garcia and Altieri 2005). Simplification of farming practices has a direct effect on increasing agricultural efficiency in terms of increased yields and profits.

However, the limitations of transgenic plants if looked at through the lens of biodiversity can be summed up as those associated with ecological processes that have an impact on operation and molding of agrosystems (Garcia and Altieri 2005). Some of the major limitations can be viewed as under:

a. The spread or acquisition of transgenes to wild or weedy relatives leading to what sometimes are termed super weeds that may lead to reduction or increase of the fitness of non-target weeds or local varieties and also selection of more herbicide-resistant and noxious weeds.
b. The evolution of resistance of insect pests to transgenic insect toxins and the accumulation of transgenic toxins, which remain active in the agricultural land.
c. The disruption of natural control of insect pests through intertrophic-level effects of the transgenic insecticidal toxins and the unwarranted effects on non-target herbivorous insects.
d. The transgenic vector-mediated horizontal gene transfer and an uncontrollable recombination that will lead to creation of new pathogenic organisms.
e. The escalation of new herbicide use in herbicide-resistant crops that lost their originally introduced resistance crops that may lead to environmental impacts including reduced weed populations and in turn plant diversity that may also result in.
f. The indiscriminate reduction of weed populations due to uncontrolled herbicide sprays in fields planted with herbicide-resistant transgenics leading to declines in bird populations that feed on or shelter in weeds or feed on the arthropods supported by weeds.
g. The reinforcement of genetic homogeneity and promotion of monocultures that would result in crops vulnerable to climate change, pests and disease.

1.3 The Impact of Transgenic Crops on Biodiversity

In the present agricultural scenario, the most direct negative impact on biodiversity is the conversion of natural ecosystems into agricultural land that has a tremendous environmental impact in terms of a significant loss of natural habitats. In overcoming this environmental hazard, transgenic crops have had a significant role to play in the past decade with their potential to increase crop yields, decreasing insecticide use, promoting the use of environmentally friendly herbicides and the facilitation of conserved tillage practices (Carpenter 2010, 2011). The efficient utilization of transgenic crops has a beneficial trade-off in terms of the requirements for lesser land to produce high yielding pest and herbicide-resistant varieties as compared to traditional crop practices that are low yielding extensive agricultural systems requiring more land and pesticide usage. This will also free more land that would otherwise be forcibly be converted to agricultural land, thus minimizing the negative impacts of biodiversity on non-arable lands. Many recent reviews have focused on both well-researched as well as hypothetical scenarios of transgenic crop cultivation related to their impact on biodiversity (Garcia and Altieri 2005; Raven 2010; Carpenter 2011; Jacobsen et al. 2013). The present review attempts to focus on an overview of the most important factors of transgenic crop effect on biodiversity and suggest some application-oriented research strategies.

1.3.1 Impact on Speciation and Impact on Traditional Crop Cultivation

One of the biggest concerns expressed against transgenic crops is their potential to reduce species abundance or the levels of genetic diversity within cultivated varieties that include traditional land races as the focus will be on a small number of high value cultivars (Ammann 2005). Initial studies conducted on transgenic cotton related to field genetic uniformity (Bowman et al. 2003) showed a significant reduction in uniformity as compared to a study conducted on conventionally bred glyphosate tolerant cotton (Sneller 2003) that showed no little impact on diversity. However, the scope of examining the consequences of transgenic varieties on diversity needs to be expanded to consider the impacts at three levels, namely, the crop, farm and landscape levels (Carpenter 2011) to accommodate all the levels of agricultural biodiversity from the gens to the ecosystems. When examined under this umbrella, it is seen that transgenic crops increase the crop diversity by enhancing underutilized alternative crops and making them widely domesticated (Gressel 2008) as seen in orphan crops such as sweet potato (Bhattacharjee 2009). In case of impacting farm-scale diversity, transgenic crops had no significant effect on non-target soil organisms and weed communities (Carpenter 2011). At the landscape level, introduction of transgenic corn, soybean and canola resulted in reduction in encroaching into non-arable lands, and also helped in environmental friendly expansions of arable lands (Bindraban et al. 2009; Brookes et al. 2010; Trigo and Cap 2006).

Further, an area-wide suppression of target pests by Bt corn and cotton led to pest management benefits in other cultivars such as soybean and vegetables (Carpenter 2011). Research over the past decade has shown that the commercial cultivation of transgenic crops had a positive impact on biodiversity through increased yields that resulted in an alleviation of pressure to encroach on non-arable land, increased use of environmentally friendly herbicides, reduction of insecticide use adoption of conserved tillage and a general increase in agricultural sustainability (Carpenter 2011). An enhanced knowledge on the part of farmers who are now better educated in agricultural practices also takes care of the fact that the purity of traditional varieties will be maintained as most of the farming community grow both traditional and improved types in the same field (Bellon and Berthaud 2004). In fact, the situation faced due to transgenic crop commercialization is no different from the times when agriculture was exposed to conventionally bred commercial crops (Ellstrand 2001). There is a mechanism in place to study the impact of transgenes in the form of an environmental impact statement requests by the USDA (Aphis-USDA 2007) that precedes the release of a new transgenic plant.

1.3.2 Impact on Natural Environment and Populations

Mathematical modeling predictions showcased an undue ability for herbicide tolerant transgenics in affecting the diversity and numbers of natural organisms that play an important role in controlling pests and diseases (Watkinson et al. 2000). The persistence of a transgenic plant released into the natural environment and any unforeseen competition with native species is dependent on the introduced transgene's invasiveness (Conner et al. 2003; Hancock and Hokanson 2001). A proper framework in assessing the stable and predictable transgene expression that enables a transgenic crop to grow better in an environment would focus on its ability to outcompete a wild species in terms of crucial factors such as reproductive success as it would be a measure of a decrease in biodiversity (Hancock 2003). Studies till date that monitored engineered traits such as insect resistance (Romeis et al. 2008) and stress tolerance (Nickson 2008) have not shown any evidence in terms of the transgenic crops invading unmanaged habitats or outcompeting wild species. Monitoring agencies mandate a strict contextual environmental risk assessment of every transgenic crop (Hancock 2003) and tiered tests are in place to assess the potential environmental risks related to fitness changes in hybrids between transgenic crops and wild relatives (Raybould and Cooper 2005). In UK, a well researched study to quantify the effects of herbicide tolerant crops such as sugar beet, maize and oilseed rape on bird and animal populations was carried out, where the impact of transgenic and conventional herbicide tolerant crop was compared. A 5 year study of 266 field trials showed a non-uniform pattern (DEFRA 2007) where in case of sugar beets and oil seed rape, the conventional varieties harbored more insects due to presence of weeds. In case of maize, there were more weeds and the late herbicide application resulted in more butterflies and bees. The differential environmental effects were attributed to new weed control strategies practiced by farmers as opposed to the mere presence of the transgenic crops (Lemaux 2009)

1.3.3 The Myth of GM-Induced Superweeds

The potential scenario of a transgenic herbicide tolerant trait introgressing into a native wild relative gave rise to the concept of a hypothetical superweed that can take over entire ecosystems and be completely resistant to existing herbicides (Chapman and Burke 2006). This challenge, though not based on fact, was not limited to transgenic crops but was first observed as a gene flow from conventional domesticated herbicide tolerant crops to its wild relatives (Ellstrand et al. 1999; Itoh 2000). This never led to the exaggerated scenarios of environmental disasters, but only limited the effectiveness of existing weed control strategies and hampered weed management options. Conventional breeders did manage the situation efficiently with strategies such as revolving dose sprays to effectively delay the evolution of both quantitative and major monogene resistance traits acquired within field populations (Gressel et al. 1996; Gardner et al. 1998). Evidence for herbicide tolerance trait tolerance from transgenic crops resulting in resistant weeds was seen in well documented studies (Watrud et al. 2004; Nandula et al. 2005; Warwick et al. 2008; Lemaux 2009). As in conventional breeding, this could be still be attributed to a single herbicide overuse (Lemaux 2009), and this was effectively overcome with developing transgenic crops that have herbicide tolerance with alternate mode of action that can be used in crop rotations to slow the resistance in weeds (Behrens et al. 2007).

1.3.4 Impact on Non-target Species

Transgenic crops could potentially impact unintended target species such as beneficial pollinators, soil organisms and endangered species and such indirect effects were seen as threats mainly from the Bt crops (Marvier et al. 2007; Duan et al. 2008; Wolfenbarger et al. 2008). However, this concern could be equally applicable to both transgenic crop fields as well as fields sprayed with conventional pesticides (Whitehouse et al. 2005; Cattaneo et al. 2006). As with most challenges in a transgenic field, the potential for the negative impact on non-target species is assessed closely monitored by regulatory agencies before commercial approval as can be illustrated with Bt cotton trials in India (Bambawale et al. 2004) and China (Pray et al. 2002; Huang et al. 2002, 2005) where a tiered approach focused on a comprehensive risk assessment related to the introduced transgenes and direct exposure to their expressed products and the indirect exposure through feeding patterns and accumulation of expressed gene products in the release environment. No significant differences were observed between conventional and transgenic fields and the studies indicated that Bt cotton cultivation had an overall beneficial effect on biodiversity as compared to regular applications of insecticides (Pray et al. 2002; Gepts 2004).

1.4 Harnessing Transgenic Technology to Promote Biodiversity

Transgenic technology has been successful in pyramiding beneficial genes into single crops (Zhao et al. 2003), introducing single genes to combat multiple stresses (Kasuga et al. 1999) and the ability to develop environmentally friendly disease resistance (Lorito et al. 1998; Emani et al. 2003). As an effective tool to increase biodiversity, transgenic technology has to now focus on identifying effective strategies both in identifying novel genes, model crop systems and effectively exploit the expanding applications of bioinformatics and systems biology.

1.4.1 Identifying Effective Experimental Model Crop Plant Systems

The identification of model plant species to study the genetic, biochemical and molecular basis of plant biodiversity is still not fully realized. Plant molecular biology has christened *Arabidopsis thaliana* as a model system due to its complete genomic sequence in the public domain, easy transformation protocols, short generation times, availability of expressed sequence tags (EST), microarray and proteomics data, and a large set of well-characterized mutants as exemplified by the *Arabidopsis* information resource (TAIR) database (http://www.arabidopsis.org). Efforts geared towards identifying model plant systems by utilizing the molecular and genetic data available on TAIR through bioinformatic analyses can be a good starting point for researchers. The successful completion of complete genomes of rice (Yu et al. 2002; Goff et al. 2002) and sorghum (Paterson et al. 2009) and more recently banana (D'Hont et al. 2012) opens the doors for analyses of specific genes as illustrated by studies made in *Arabidopsis* (Denby and Gehring 2005). A comprehensive database for model experimental plants among edible crops, forest species, pharmaceutically important plants and biofuel crops aimed at data related to transformation protocols, ESTs, microarray, experimental mutants, transcriptome and proteome data in line with the TAIR database will be an effective resource for breeders aiming to develop novel plants to preserve biodiversity.

1.4.2 Plant Databases for Targeted Gene Discovery

Existing databases such as TAIR (http://www.arabidopsis.org), Gene Ontology (http://www.geneontology.org), Plant GO slim (http://www.geneontology.org/GO.slims.shtml) and the more recent TRY (http://www.try-db.org) with over 3 million trait records for 69,000 plant species with the integrated whole genome profiling information can be utilized towards concerted efforts aimed at identifying a vast number of target genes related to preserving plant biodiversity. The identified target genes with crucial molecular functions related to preserving and enhancing plant biodiversity can then be evaluated for their biotechnological potential by genetically engineering them into popular cultivars and forest species.

1.4.3 The World of Small RNAs

A more recent development in the form of the discovery of microRNAs involved in an array of molecular processes in popular crop plants such as rice (Li et al. 2010) and sorghum (Zhang et al. 2011) is opening an entirely new and effective field of plant molecular research (Nelson et al. 2003). Techniques are now available in designing and silencing miRNAs for various traits across plant species (Ossowski et al. 2008; Warthmann et al. 2008; Schwab et al. 2006) and a dedicated web site makes the technology readily accessible (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi). Researchers can effectively use the technology to design artificial 21-mer microRNAs (amiRNAs) that can be genetically engineered and function to specifically silence single or multiple genes of interest across plant species according to the previously determined parameters of target gene selection.

1.5 Conclusion

Charles Darwin in his monumental work *The Origin of Species*, focused on biodiversity to unravel the biological mechanics behind the rich variety of life forms on our planet (Darwin 1859). Darwin attributed the evolution of diverse species on earth to the ability of plants and animals adapted to their environment to breed and pass on their characteristics to their offspring. In the revolutionary conclusion to his classic theory, Darwin reflected on the crucial principle of the importance of relationships between species and contemplated an “entangled bank” where various life forms live in unison. This unraveled the fact that no species including our own *Homo sapiens* can exist in isolation from other living things. Every species on earth is dependent on natural processes for its own survival and in doing so contributes to the natural balance of the environment that translates into the very survival of our planet. We as human beings can thus be the agents of change to the preservation as well as degradation of the rich biodiversity on our planet. With the ever growing field of plant biotechnology that has a diverse array of technological applications to choose from, a planned contextual strategy to best utilize the available state of art techniques will richly benefit future researchers and their studies. The planned research strategies will help fulfill our existence as a human race in being the very agents of change that are responsibly preserving and enhancing the rich plant biodiversity to benefit planet earth, while systematically exploiting its resources to better our lives.

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