ENVIRONMENTAL CONCERNS AND POSSIBLE STRATEGIES TO REDUCE THE POTENTIAL RISKS OF PLANT MOLECULAR FARMING - REVIEW

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Abstract

The production of useful recombinant proteins was perceived since the existence of genetic modification technologies, in so doing, transgenic plants have been materialized. Transgenes flow remains as one of the global concern in the area of plant molecular farming that deteriorates the environment. This review aimed to evaluate the potential risks of plant molecular farming on the environment and their possible controlling strategies by assessing secondary data. Various sources of literatures have stated that the possible environmental risks of molecular farming rely on genetically engineered organisms are including but not limited to creating new or more vigours insect pests and pathogens; exacerbating the effects of existing pests via hybridization with related transgenic individuals; harm to non target species such as soil organisms, non- pest insects, birds and other animals; disruption of biotic communities including agroecosystems; irreversible loss or changes in species diversity or genetic diversity within species. These potential risks of molecular farming could harm the well-being of humans, animals and the environment at large. A number of different interdependent options such physical and biological approaches have been put in place to reduce food/feed chains contamination or environmental pollution due to PMF. Some the most important gene flow barriers are production of proteins by cell suspension culture, chloroplast transformation, cytoplasmic male-sterility, sexually incompatible crops, seed terminator, tissue specific expression technology, labs filters, and greenhouse/glasshouse and isolation distances. Some sources indicated that, the overall acceptability of molecular farming applications seemed less appreciable by the society. It is suggested that the potential environmental risks from the plant molecular farming can be reasonably minimized by controlling of the gene flow from the transgenic to conventional plants.

Keywords: Plant molecular faming, GM crops, Environmental concerns, Environmental risk reducing strategies

Összefoglalás

A hasznos rekombináns fehérjék előállítása a genetikai módosítási technológiák kifejlesztése és a transzgénikus növények megjelenése óta folyik. A transzgének áramlása azonban globális problémát jelent a növényi molekuláris gazdálkodásban, mivel rombolhatja a környezetet. Jelen tanulmány célja a növényi molekuláris gazdálkodás potenciális környezeti kockázatainak értékelése másodlagos adatok elemzésével, valamint javaslatot tesz a lehetséges szabályozási stratégiákra is. Irodalmi források szerint a genetikailag módosított szervezeteket használó molekuláris gazdálkodás környezeti kockázatai – nem kizárólag – új vagy erősebb kártétellel rendelkező rovarkártevők és kórokozók megjelenését; a meglévő kártevők kártételeinek súlyosbodását; a nem célzott fajok károsodását; a biotikus közösségek, köztük az agroökoszisztémák megzavarását; a fajok sokféleségének vagy a fajokon belüli genetikai sokféleségnek visszafordíthatatlan változását is előidézhetik. A molekuláris gazdálkodás ezen lehetséges kockázatai veszélyt jelenthetnek az emberek, az állatok és a környezet egészére nézve egyaránt. Fizikai és biológiai elhatárolási megközelítéseket vezettek be az élelmiszer/takarmányláncokban a növényi molekuláris gazdálkodás miatt megjelenő szennyeződések és a környezetszennyezés csökkentése miatt. A fehérjék termelése sejtszuszpenziós tenyésztéssel, kloroplaszt transzformációval, a citoplazmatikus hímsterilitás, a nemi szempontból inkompatibilis növényfajok használata, a terminátor és a szövetspecifikus expressziós technológiák alkalmazása, a laboratóriumi szűrők, és az izolációs távolságok megtartása jelentik a génáramlás megakadályozásának legfontosabb eszközeit. A tanulmány rámutat arra, hogy a molekuláris gazdálkodás alkalmazásainak általános társadalmi elfogadhatósága kevésbé érezhető. Lehetséges megoldás, hogy a molekuláris gazdálkodás környezetre gyakorolt negatív hatásait főként a génáramlás – GM növényből a nem génmódosított növényekbe történő mozgásának – ellenőrzésével lehetne minimalizálni.

Kulcsszavak: növényi “molekuláris gazdálkodás”, GM növények, környezeti aggályok, környezeti kockázatokat csökkentő stratégiák

Introduction

Since gathering ear till today plants have been a potential source of medicinal drugs. In line with (Grifo et al., 1997) report nearly 57% of the well-studied drugs had exhibited a minimum of a single main active ingredient initially purified from plant source. Winslow & Kroll (1998) had reported also that about one fourth of medicines usage was sourced from plant origin. Plant molecular farming uses either whole organisms, various plant parts or cultured cells as bioreactor, and that encompasses genetic modification of agricultural products for the production of commercially valuable and pharmaceutical oriented proteins and chemicals on large scale and at low costs (as reviewed by Tarinejab & Rahimi, 2015). It is also believed that this plant based technology can potentially solve the current demand for the biomedicine (Ahmad, 2014). In this regard, scholars could select suitable host plants to be used in the plant molecular farming biotechnological program. As a result, numerous host plants' selection criteria had been studied eventually the most informative and profitable ones are identified such as total biomass yield, ease of transport, storage attributes, and value of recombinant proteins, life cycle, required area, maintenance costs, labor availability, edibility, and cost of the final product (Fischer et al., 2004; Schillberg et al., 2005). To this end, some of the lists of crop that have been manipulating in molecular farming are tobacco, canola, potato, safflower, alfalfa, lettuce, soybean, rice and maize. Among these crops rice and maize has been recognized as the most suitable and the later had exhibited the highest biomass yield from the domain of food crops with soften transformation and maximum final product production (Ramessar et al., 2008). Generally plant based pharmaceuticals are found to be safer, storable, less costly and produced in bulk (Ahmad, 2014). Despite its importance and remarkable insights there exist two main classes of risks of molecular farming. One affects human beings and other harms the environment, and other organisms; it was evident that grain crops found to be the most suitable for this technology, but it is full of controversy the grain transformation using agrobacterium in the production of pharmaceutical proteins; the immune system can be incapacitated the medicines produced in plants and rather be the initiator for allergic reactions (Hout, 2003). Similarly, (Tarinejab & Rahimi, 2015) had reported that the use of transgenes could impose a higher degree of replication and transmission of genes, toxic recombinant proteins deposition in the ecosystem which in turn led to food web contamination and increased costs of remedies. This review paper briefly assessed some progresses of molecular farming with a central focus on environmental associated risks and the complementary strategies to reduce the possible negative impacts of the molecular farming in the ecosystem and human health aspects.

Table 1. Acceptability of plant molecular farming (PMF) applications as revealed by a case study in Canada, Alberta, University of Calgary in 2005.

Applications	**Fully acceptable**	**More acceptable**	**Less acceptable**	**Unacceptable**
Interleukin in tobacco	8	25	13	2
Edible vaccines (Norwalk in potatoes)	10	25	11	2
Gastric lipase in corn for cystic fibrosis	6	26	15	1
Trypsin grown in corn for industrial uses	1	14	21	11
Bioplastics grown from corn	6	21	14	6
Overall impression of PMF	3	29	10	6

(adapted from Einsiedel and Meldock, 2005)

Potential risks of plant molecular farming in the environment

Both the potential benefits of plant molecular faming and it’s possible influence reaches to human biengs, animals and the extended environment too; and the target of assessment for human, animals and environmental safety issues become a priority due to their exposure to the plant molecular faming products (Breyer et al., 2009). A case study shown that the overall acceptability of plant molecular farming application was found very low impressive to the end users (Table 1). It is evident that the active ingredients of the pharam plants could enter to the water bodies and even eaten by animals which eventually affect these entities, and this could result desensitization of the vaccine so that it would stop its functionality as well (internet1).

Such types of environmental concern issues due to plant molecular faming had manifested in the year 2002 in United States of America and recorded as first public incident. In the same year, transgenic maize was grown in the field of soybean to harvest trypsin the pharmaceutical active ingredient followed by soya production, however, 13,500 tons of soybean produce was damped because it was found contaminated by the prior plantation. According to Fernandez et al. (2014) together with different regulatory agencies reached into consensus as any regulatory review should encompass environmental concerns such as weedy nature of the crop, out-crossing ability of the transgenic crops with their wild relatives or cultivated crop species and influence on non-target living things.

One of the most threating burning issues of plant based pharmaceuticals are poising of the food chain. So far, studies had revealed that crossing of conventional genetic materials with transgenic pollen sources be it by using the same harvesting equipment, process without precise decontamination, growing crops adjust to transgenic crops or ignoring of the soil from proper decontamination ahead of non-transgenic cropping practiced (Rigano and Walmsley, 2005). To this end, the ultimate seed bank could be even distorted as a resultant of contaminated non-genetically engineered crops and weeds (Mallory-Smith & Sanchez Olguin, 2011). In a similar fashion and even more intense herbicide resistance genes could transfer from crops to weeds and posed difficulty to control these weeds (Gressel, 2015). According to (Breyer et al., 2012) ingestion of the recombinant proteins and/or the transgenic plant itself could cause a potential skin or eye problem and allergy primarily in children. The problems associated with plant molecular farming are not imagery, rather it could be demonstrated by these two examples- the case of ProdiGene and StarLink concerns (Murphy, 2007). Originally, ProdiGene is a vaccine used to prevent bacteria-induced diarrhea in pigs produced from a transgenic corn, though it was non-toxic to human but it was strictly advised not to be a part of the human food chain

(Hileman, 2003). Similarly, millions of tons of non-transgenic corn was contaminated by the StarLink transgenic across the United States. The cost of recollection and dumping of the contaminated corn by Aventis was estimated $ 500 million (Murphy, 2007). It is not likely to be true, however, a gene could follow from transgenic crop to noxious weed then after this weed to another non-genetically engineered crops. Along this line, these contaminated weeds could harbor that transgene permitting expansion to non-engineered crops. The most important environmental concerns about the use of GM crops for various purposes are: increased use of toxic pesticides, unforeseen consequences (Pleiotropy) and genetic contamination (internet2). Moreover, the possible risks of genetically engineered organisms to the environment including but not limited to creating new or more vigours insect pests and pathogens; exacebating the effects of existing pests via hybridization with related transgenic individuals; harm to non target species such as soil organisms, non- pest insects, birds and other animals; disruption of biotic communities including agroecosystems; irreversible loss or changes in species diversity or genetic diversity within species (as reviewed by Snow et al. 2005). As a worse case scenario, mutation and extinction of species may become a dominant event and cause abnormalities within the large biological entities (Godheja, 2013). However, they are some still argue that, as tittle is known about the drawbacks of plant molecular farming to the environment, and human health since the technology is relatively new, and most of the research works are strictly laboratory based with a few filed trials (Hout, 2003). These interrelated plant molecular farming concerns need due attention starting from their production technology selection up to proper usage to make them user and eco-friendly so as to ensure sustainability.

Strategies to minimize the potential risks of plant molecular farming

So far, three dominant entries of transgenes into the ecosystem have been identified. These ways of spread are volunteer plants (Michael et al., 2010), pre and during harvesting shattering of seeds and cross-pollination with the adjust crops (Gressel, 2015). The tradeoff of plant molecular farming hits the environment, human welfare and at large the economy, this calls the development of mitigation measures and implementing of strategic controlling means to the spread of transgenes (Clark & Maselko, 2020). These potential risks of blending and pollution of GM crops utilized in plant molecular faming associated with agriculturally vital crops could be minimized by using non-food/forage crops of PMF. In this respect, various strategies such as production of recombinant proteins by cell suspension culture in bioreactors, restrict physical agronomic confinement, post-harvest field monitoring and sanitation, use of late maturing or early maturing cultivars at the different time period to ensure harvesting before or after other crops intended for food /feed and processing are among the frequently used ones (Obembe et al., 2011; Spok et al., 2008). Moreover, contaminating gene flow can be blocked by implementing various facilities like greenhouses, glasshouses, hydroponics; and biological advancements such as chloroplast transformation, cytoplasmic male-sterile transgenic plants, creating of sexually incompatible crops, seed terminator, parthenocarpy and tissue specific expression technology (Valkova, 2013; Salehi, 2012). To harvest the maximum benefit of plant molecular farming without or with minimum environmental drawbacks, it is highly recommended synthesizing scientific and regulatory risks assessment, and management strategies and standards too (Jouzani & Tohidfar, 2013).

Regulatory frameworks

There exists regulatory frameworks and guidance to plant molecular farming, and here the case of the United States and the European Union is briefed as below. The Coordinated Framework for Regulation of Biotechnology has come to existence for the first time in 1986. The agency called Animal and Plant Health Inspection Service has been responsible for regulating the plant molecular farming (PMF) production process while the Food and Drug Administration (FDA) targets the end products safety and pharmacological aspects. For example the use and cultivation of GM crops outside the delineated and predetermined growing sites need an authorization (internet 3) In the European Union GMO regulatory frameworks have been formulated. The Directive 2001/18/EC has been in account for regulating the boundless activities either for experimental or commercial conscious release of GM crops (EC, 2001). This Directive 2009/41EC has also allowed the limited use of GM micro-organisms considering their likely harmful outcome for human health and the environment with due emphasis to their accident preventive and control of wastes (EC, 2009). Recently some amendment was made by the Directive 2015/42 EU, and it stated that member states could cultivate GM crops by employing suitable measures to get rid of possible cross-border contamination into neighboring member states where cultivation of GM crops is prohibited (EU, 2015). From these directives and regulatory frameworks one can understand that GM based plant molecular farming technologies remain as one of the potential concern to the ecosystem.

Physical and biological transgene flow mitigation approaches

Gene flow is a natural process in which plant populations exchange genes due to the crossing of gametes at varying frequencies (Cerdeira & Duke, 2006). This happens within the closely related and rarely between species. Following this path, some persuading confirmations of transgene flow has been realized for example in cotton, maize and soybean (Baltazar et al., 2015; Dong et al. , 2015; Londo et al., 2011). Nearly all transgenes have been gotten away into their partner and wild relatives. In spite of the fact that gene flow changes between species, crops and environmental zones/environments but intraspecific gene flow (> 10%) is not an exceptional in adjoining populations. While in outcrossing species, 1% gene flow at thousand meters' confinement is not unordinary, and size is indeed higher than the mutation rate (Rizwan et al., 2019). Therefore, this global concern needs sound mitigation approaches besides to regulatory frameworks and appropriate production of molecular farming, there are a number of different interdependent alternatives grouped as a physical and biological gene flow mitigation approaches that can reduce food/feed chains contamination or environmental pollution due to PMF.

Table 2. Some selected compatible strategies for minimizing the potential risks of PMF to the environment.

Types of approaches	Specific Cases	Purpose-Examples	References
Physical containment	Plastic tunnels and greenhouse	Production of biopharmaceutical alfalfa for therapeutic proteins	(Zayon & Flinn, 2003)
	Delineated land	To eliminate the risk of gene flow to non-farming plants and wild relatives	(Howard & Hood , 2007)
	Isolation distance	Minimum gene contamination-via gene flow	(Linder et al., 1998)
	Non-transgenic trap plants	Reduced gene contamination-due gene flow	
Biological blockage	Plastid transformation	The production of vaccine antigens and pharmaceutical	(Daniell, 2006)

Greenhouse/glasshouse meshes, filters in the laboratories and isolation distances in the field serve as physical barriers. It seems less likely to record non-success story of physical containment in the lab or greenhouse, however, which is not the case in the field. In line with (Fox, 2003) report traces of transgenes from previously cultivated ProdiGene harboring maize were found on small magnitude of maize leaf trash adhering to the following crop. Biologically, uncontrolled hybridization can be reduced to the possible minimum tolerable rate by mismatching the relative flowering times of GM and non-GM crops. In this way, gene flow would be prevented whenever the anthers pollinate pistils before flowers open (Gruber & Husken, 2013). Some of the most powerful physical and biological strategies to reduce the potential risks of plant molecular farming to the environment are listed below (Table 2).

Conclusion and future prospects

Historically, plants have been a potential source of medicinal drugs. Plant molecular farming uses either whole organisms, various plant parts or cultured cells as bioreactors, and produce pharmaceuticals at large scale and low costs. Despite the current technological developments and the potential merits of plant molecular farming for the betterment of mankind there are also uncertainties associated with it. As a matter of fact, genetically engineered crops based plant molecular farming is found to be capable of contaminating the environment, non-GM plants, wild relatives and even weeds this eventually led to food/feed chain contamination. It is also evident that the active ingredients of molecular farming products could enter to the water bodies, and even be eaten by animals which in turn affect these entities, and this could result desensitization of the vaccine so that it would stop its functionality. The impact of plant molecular farming in the environment, biodiversity richness, human health, and the economy could be kept down via controlling the gene flow of the transgenes. In doing so, it is suggested that regulatory frameworks shaped the appropriate production and utilization of molecular farming applications. To this end, there are also a number of different interdependent options assembled as a physical and biological approaches that can reduce food/feed chain contamination and environmental pollution due to PMF. Specifically, gene flow can be prevented by various physical and biological barriers. On one hand, plastic tunnels, greenhouses, delineated land, isolation distances and non-transgenic trap plants serve as physical barriers against gene flow. On the other hand, plastid transformation, cytoplasmic-male sterility, seed terminator technology, transient expression, cell-suspension culture, and creating sexually incompatibility crops and some others have been practicing to reduce the potential risks of plant molecular farming to the environment at large. The sector still remains challenging and suspicious. It is therefore attention is needed in implementing all possible advancements in the field enabling reduction of gene flow into the agricultural production systems, and the environment at large. Equally, unbiased risk assessment to evaluate the merits and demerits of new traits to the environment will remain instrumental to the efficient application of plant molecular farming.

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