INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0925/2019/22–6–1293–1304
DOI: 10.17957/IJAB/15.1201
http://www.fspublishers.org

Review Article

Characterization of Genetically Modified Plants Producing Bioactive Compounds for Human Health: A Systemic Review

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Abstract

Increasing knowledge on plant biotechnology, nutrition and medicine has altered the concepts regarding food, health and agriculture. Researchers in medical biotechnology as well as plant biology are recommending the application of plant systems, their products such as phytoceuticals, foods and phytotherapy to perk up human health along with disease prevention and treatment. Plants derived pharmaceuticals offer numerous advantages over other techniques such as mammalian cell culture methods *etc*. These modern trends have highlighted potential targets for the blooming pharma industry based on plant biotechnology. Most early studies, as well as current work, focus on crops as well as non-crop transgenic plants expressing several advantageous proteins that can be extracted, processed and used in the treatment of different diseases. The major part of public opinion is positive regarding the competence of biotechnology for improving life quality and standards. But unfortunately, due to some ambiguities in consumer's mind, phytoceutical production from transgenic plants is a bit ignored field both at researcher and consumer end. This review tried to put on view and differentiate the production of phytoceuticals from transgenic plants concerning human health. Readers will find twenty years of progress on phytoceuticals and their relatedness to reported human disease studies as well as trials. It also covers a comparison of different techniques to distinguish the production of phytoceuticals from transgenics and issues along with existing regulations for their safety and commercialization.

Keywords: Biotechnology; Cost; Efficacy; New Medicines; Risks; Transgenic plants

Introduction

Our the years, pressure upon natural resources and their utilization for human benefits is increasing linearly. Either it is food insecurity or environmental constraints, humans are being affected in one or the other way. Natural resources have a question mark upon their capacity to cater to human demands. Besides, it is expected that the world population would reach 8.5 billion by 2025 (Ahmad *et al.,* 2012). Today, other than food security across the globe, food deficiency and malnutrition are serious issues demanding immense control as well as regulatory measures. In the Asian region, a significant segment of the population is facing health challenges, developmental problems and growth anomalies (Batabyal *et al.*, 2019). The nexus between human health and disease is pointing out the need for producing medicine with high efficacy and low cost along with the application of technology to improve human life.

For centuries, plant products are in human use due to their unique properties and combination for the treatment of different ailments (Shinwari and Qaiser, 2011; Noman *et al.*, 2013). Various plant species are famous for making drugs in Indian and Greek medicine systems (Reski *et al.,* 2015). Different angiosperms and gymnosperms are the sources of

To cite this paper: Aqeel, M., A. Noman, T. Sanaullah, Z. Kabir, M. Buriro, N. Khalid, W. Islam, M. Qasim, M.U. Khan, A. Fida, S. Fida, M.A. Akram and S.U.R. Sabir, 2019. Characterization of genetically modified plants producing bioactive compounds for human health: a systemic review. *Intl. J. Agric. Biol*., 22: 1293–1304

traditional plant-derived pharmaceuticals. Now, scientists are applying technology on non-traditional plants like algae, mosses etc. to produce multipurpose products such as vaccines, foods and fuels (Kumar *et al.,* 2013; Reski *et al.,* 2015). The developments in biotechnology have highlighted the sustainability, novel possibilities and opportunities for improving the qualitative and quantitative plant attributes and products (Trivedi and Nath, 2004; Sun, 2008; Liu *et al.,* 2017; Noman *et al.,* 2018). Plant biotechnology offers new strategies to develop transgenic crops with high yield, stress tolerant, resistance to diseases, insects and herbicides (Noman *et al.,* 2017a; Hussain *et al.,* 2018; Khan *et al.,* 2018; Islam *et al.,* 2018). Moreover, traits like nutritional aspect, medicine and biofuel production have been focused on human benefits (Maughan *et al.,* 2018). All of these traits involve multiple gene operations (Tiwari *et al.,* 2009).

Prior research works suggest that the need for natural medicines is rising day by day (Babura *et al.,* 2017; Paul *et al.,* 2017). Phytoceuticals are hovering to be a chief viable product of plant biotechnology (Zafar *et al.*, 2013; Fischer *et al.*, 2004). Phytoceuticals from autotrophs would be cheaper and easily storable. Therefore, these would be in access to all and sundry. Limited attention on the production of few vaccines or insulin is utterly inadequate and disappointing to some extent. By adopting diverse genetic engineering (GE) techniques, plant biotechnologists are trying to augment phytoceutical genesis and production of nutrient-rich plants (Joh and VanderGheynst, 2006; Linnhoff *et al.,* 2017). Their advantages such as production capacity, merchandise, safety, easy storage and supply cannot be met by any present business method (Table 1; Paul and Ma, 2011; Svennerholm, 2011). They also offer favorable prospects to provide low-cost drugs and vaccines to the developing world.

Although transgenic plants have been developed and got fame yet faced legal, political and social pressures, *e.g*., production of allergenic foods (Ashraf and Akram, 2009). Despite increasing production of genetically modified (GM) plants, medicinal aspect of transgenic plants has not been addressed according to its value (Maughan *et al.,* 2018). Though crop growth, stress tolerance and yield enhancement are obligatory, it is unfair to leave all other plant attributes (Stoger *et al.,* 2005). Likewise, in spite of unparallel benefits, the commercialization of phytoceuticals is overshadowed by some factors like uncertain regulatory systems *etc*. This article broadly discussed the developments in biotechnology for producing transgenic plants with enhanced phytoceutical capacity as well as nutritional status. Additionally, critical views, existing regulations and commercialization issues of medicinally valuable transgenic plants have also been discussed.

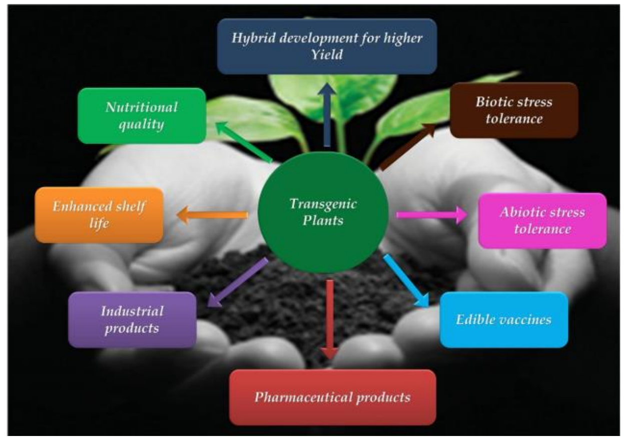

Fig. 1: Development of transgenic plants has revolutionized plant attributes for Human benefits. Application of Biotechnological techniques have improved production of trangenic plants with particular emphasis on enhancement of attributes such as nutritonal quality, stress tolerance *etc.* Biotechnology is helping pharmaceutical industry in developing novel methods and improving existing ones for production of pharmaceutical products

Nutrient Status Improvements in Transgenic Plants: Cure through Food

For human beings, nutrients from foods play an essential role in maintaining regular metabolism (Zhao, 2007; Maughan *et al.,* 2018). Plant improvement by biotechnological techniques has come on front as a doable strategy for enhancing food quality by improving constituents like proteins, carbohydrates and vitamins (Fig. 1 and Table 2; Twyman *et al.,* 2003; Noman *et al.,* 2016; Babura *et al.,* 2017; Paul *et al.,* 2017). Due to recent advancements in medical and nutrition sciences, health benefits from natural products has won favour from health care professionals and public as well. Innovative ideas like nutraceuticals, phytoceuticals, and phytotherapy have encompassed this trend (Bagchi, 2006; Maughan *et al.,* 2018). Health-promoting properties of plant-derived products have been proved comparatively effective in reducing disease reduction.

Plant bioactive products and nutrients with common benefits for human health may become closer to a sustainable diet, medicine or sometimes overlap with phytomedicines. Nutritional inadequacy, along with deficiency, is a daunting challenge for developing and underdeveloped realms (Linnhoff *et al.,* 2017). For instance, deficiency of vitamin A impairs human vision and can also cause maternal mortality. Today, need is to have therapeutic food items or natural dietary supplements that play purposeful roles such as well-being, health maintenance and modulation of immune functions in humans (Lee *et al.,* 2011). Globally, rice (*Oryza sativa* L.) is used as a staple food. Development of human lactoferrin and lysozyme in transgenic rice is also a great accomplishment (Paul and Ma, 2011). Golden rice variety is now able to accumulate a high quantity of provitamin A (Chassy, 2010; Linnhoff *et al.,* 2017). Health-promoting properties of plant-derived products have been proved comparatively effective in reducing disease reduction.No technology other than plant biotechnology has appeared

Table 1: Biotechology based pharmaceutical industry have produced highly efficient drugs from plants and animals. This table presents different proteins that are being widely used for treatment of diseases

Proteins	Category	Use against/as	Plant							References
			Zea mays	*Medicago sativa*	*Lactuca sativa*	*Solanum tuberosum*	*Malus domestica*	*Nicotiana tabacum*	*Oryza sativa*	
LTB	Vaccine	Diarrhea	■							Chikwamba *et al.* (2003)
Gastric lipase	Enzyme	Pancreatitis	■				■			Elbehri (2005)
Hbs Ag	Vaccine	Hepatitis B			■	■				Pniewski *et al.* (2017)
F protein	Vaccine	RSV		■						Lau and Korban (2010)
Norwalk virus CP	Vaccine	Norwalk Virus Infection				■				Tacket *et al.* (2000)
Human serum albumin		Liver cirrhosis						■		Peter *et al.* (1990)
Cholera vaccine	Vaccine	Cholera						■		Elbehri (2005)
Rabies virus glycoprotein	Vaccine	Rabies						■		Roy *et al.* (2010)
Insulin	Vaccine	Diabetes mellitus							■	Xie *et al.* (2008)
Lactoferrin	Dietary protein	Antimicrobial	■							Daniell *et al.* (2001)
Hemagglutinin protein	Vaccine	Measles virus						■		Webster *et al.* (2005)
Tetanus vaccine antigen	Vaccine	Tetnus						■		Tregoning *et al.* (2004)
CaroRx	Antibody	Dental caries						■		Elbehri (2005)
Human somatotropin	Hormone	Growth						■		Staub *et al.* (2000)

immensely useful in elevating the crop nutritional levels (Linnhoff *et al.*, 2017). During the last two decades, many examples of nutritional status improvement have been set, *i.e*., raised lysine level in cereals and high methionine content in legumes. Increased level of previously deficient constituents like vitamins and methionine in crops corroborate the outcomes of sophisticated biotechnological techniques. Initially, transgenic rice containing four *Narcissus* and *Erwinia* genes was developed (Ye *et al.*, 2000). Supporting evidence from the earlier studies and similarities between findings advocate the successful implication of biotechnology for pharmaceutical production. The success of biotechnology can be revealed from the report of Paine *et al.* (2005). According to them, people who consume 75 g of golden rice each day routinely make them prone to have enough provitamin A. With improved understanding of ascorbic acid biosynthesis and metabolism, GM vegetables with better vitamin C contents have been focused (Chen *et al.*, 2003). Vitamin C amount can be amplified by amending its recycling in plants. An excellent explanation of this finding has been successfully performed by generating transgenic maize (*Zea mays)* line presenting 100 fold increments in vitamin C levels. It is also interesting to note that the dairy starter bacterium possesses the potential to produce both folate (vitamin B11) as well as riboflavin (vitamin B2) (Burgess *et al.*, 2004). Therefore, it can be assumed that the engineered *Lactococcus lactis* strains may produce riboflavin or folate more than our expectation. So, this gives us an opportunity to get rid of synthetic vitamins. In *Arabidopsis thaliana* leaves, expression of *HGGT* from barley produced 10 to 15 times more vitamin E antioxidant contents (tocotrienols and tocopherols) (Babura *et al.,* 2017). Likely, over-expression of the same gene in maize seeds led to 6 fold augmentation in tocotrienol and tocopherol level (Cahoon *et al.*, 2003; Babura *et al.*, 2017). The vitamin overproducing crops, *e.g*., Soybean (*Glycine max)* and barley (*Hordeum vulgare)*, will not only enhance the dietetic worth of foods but these can make such foods as premium medicines served to vitamin-deficient people.

The present study raises the possibility that nutritional increments in plants further support the idea of treating human diseases in a safe way, *e.g*., Golden mustard (*Brassica juncea*) has been launched by following the same path (Vemuri *et al.,* 2018). In tomato (*Solanum lycopersicum*) and other fruits, lycopene is a persuasive antioxidant acting as an aegis against prostate and other cancers. It also inhibits tumor growth in some animals. Additionally, GM tomato contains a higher amount of flavonols and flavones (Schijlen *et al.*, 2006; Yoo *et al.*, 2017). Identification, isolation and incorporation of genes encoding lycopene are possible through biotech tools. Introduction of these genes in other food plants will not be less than a miracle. Its consumption will improve health and reduce risks (Yoo *et al.,* 2017). Likewise, organosulfur compounds in garlic and onions can be exploited for multifunctional benefits to humans (Grusak, 2005). Uplifting the essential amino acids levels in staple foods and other plants has been considered for several years (Windle, 2006). Related patents for GM crops with high storage proteins bearing good essential amino acid sources have been issued. Mounting eatable nutrients in plant-derived food is a fundamental tactic to boost mineral nutrition (Ma *et al.*, 2005; Zhao, 2007; Linnhoff *et al.*, 2017). Calcium, zinc, and iron in phytate or oxalate forms could drop dietary minerals bioavailability and restrain absorption of these minerals by human beings. Using biotechnology, altering crops and

Table 2: Phytonutrients perform specific biological functions for human health. Such nutrients are taken from the plant-based diet but chiefly refer to nutrients working as modifiers of physiological functions

Plants	Imroved nutritional status																References
	Essential Amino Acids & Proteins				Carbohydrates/Lipids /Fatty acids				Vitamins				Minerals				
	Lysine	Methionine	Tryptophan	others	Starch	Glucose	Fatty acids	Others	A	B	C	Others	Calcium	Iron	Zinc	Others	
Beta vulgaris						■											Smeekens (1997)
Brassica juncea																■	LeDuc *et al.* (2004)
							■										LeDuc *et al.* (2004)
Daucus carota												■	■				Park *et al.* (2009)
Glycine max				■													Rapp (2002)
								■									Yu *et al.* (2003)
	■		■														Galili *et al.* (2002)
Gossypium hirsutum							■										Liu *et al.* (2002)
Lactuca sativa													■				Park *et al.* (2009)
														■			Goto *et al.* (2000)
Lupinus albus		■															White *et al.* (2001)
Lycopersicon esculentum								■									Rosati *et al.* (2000)
Oryza sativa							■										Anai *et al.* (2003)
									■								Paine *et al.* (2005)
										■							Storozhenko *et al.* (2007)
														■			Johnson *et al.* (2011)
															■		Lee *et al.* (2011)
				■													Katsube *et al.* (1999)
Solanum lycopersicum											■						Bulley *et al.* (2012)
										■							Storozhenko *et al.* (2007)
Solanum tuberosum		■															Zeh *et al.* (2001)
						■											Hellwege *et al.* (1997)
								■									Hellwege *et al.* (2000)
					■												Lukaszewicz *et al.* (2004)
									■								Diretto *et al.* (2007); Lopez *et al.* (2008)
											■						Bulley *et al.* (2012)
													■				Park *et al.* (2005)
				■													Chakraborty *et al.* (2000)
Sorghum bicolor	■																Zhao *et al.* (2003)
Triticum aestivum									■								Cong *et al.* (2009)
Zea mays	■	■															Lai and Messing (2002)
							■										Young *et al.* (2004)
						■											Caimi *et al.* (1996)
								■									Yu *et al.* (2000)
									■								Aluru *et al.* (2008); Zhu *et al.* (2008)
											■						Naqvi *et al.* (2009)
														■			Drakakaki *et al.* (2005)
				■					`								Young *et al.* (2004)

vegetables for lowering the levels or removing phytate and oxalate might swell the mineral availability and absorption.

Transgenic Plant-based Antigens

Transgenic plants have been widely adopted for the production of important pharmaceuticals (Rybicki, 2018). In the 1990s, *HBsAg* DNA was introduced in *Nicotiana* plants through *Agrobacterium*-mediated transformation. The analysis of isolated *HBsAg* from transgenic *Nicotiana* was found corresponding to human serum *HBsAg* (Mason *et al.*, 1992). This achievement revolutionized the thoughts and scientists focused on biotechnological approaches to combat different diseases (Rybicki, 2018). Based on similar experimentation, parallel immunogenicity to yeast-derived vaccines in mouse has exhibited plants based vaccines (Thanavala *et al.*, 1995; Thanavala and Lugade, 2010). Likewise, *HBsAg* in *Nicotiana*, transgenic potato (*Solanum tuberosum)* expressing *HBsAg* also presented high-level immunogenicity in mice (Richter *et al.*, 2000; Thanavala *et al.*, 2005). Many existing studies in the broader literature have examined *HbsAg.* Meanwhile, some group of researchers targeted the route of administration for plant-derived antigens. Kapusta *et al.* (1999) conducted experiments by using transgenic lettuce harboring *HBsAg* expression plasmids. Orally given transgenic lettuce showed positive response in comparison to a vaccine. A first successful trial in a human was conducted against ETEC (Enterotoxigenic *E.coli).* The volunteers were fed with transgenic potato expressing LTB (*E. coli* heat labile enterotoxin B subunit). Amazingly, neutralizing antibodies were developed in more than 90% volunteers, while more than half of volunteers exhibited mucosal response Tacket *et al.* (2004). Similarly, Arakawa *et al.* (1998) recorded positive observations in transgenic potato expressing *CTB* (B subunit of the cholera toxin). In 2000, success was achieved when scientists became able for the first time to use an edible vaccine, *i.e*., RSV fusion (F) protein to fight the respiratory syncytial virus (Sandhu *et al.*, 2000). With this accomplishment, researchers were infused with great zeal and the first decade of the 21st century is marked with many success stories. Therefore, in the light of reported results, vaccines for other diseases were also prepared and confirmed by biotechnologist. LTB-expressing transgenic tobacco (*Nicotiana tabacum*) presented capacity as vaccine or booster vaccine against enterotoxigenic *E. coli* and cholera (Qadri *et al.,* 2005; Svennerholm, 2011). A thorough look into literature reveals that *rHBsAg* expression in banana (*Musa paradisiaca)* as an edible vaccine can be a viable option in opposition to HBV (Hepatitis b Virus) infection (Richter *et al.,* 2000; Elkholy *et al.,* 2009; Pniewski *et al.,* 2017). Oral administration into mice, Protective antigen As16 boosted As16-specific serum antibody response that led to low lungworm burden (Matsumoto *et al.,* 2009). Furthermore, plants used as a vegetable were explored for their potential to yield vaccines and antigens (Pniewski *et al.,* 2017). *CTB::VP60* pentameric protein a derivative from *Pisum sativum* sheltered rabbits against RHD virus (rabbit hemorrhagic disease) (Mikschofsky *et al.,* 2009). Transgenic tobacco possessing MV-H, measles virus, developed antibodies manifold more than needed for human protection (Huang *et al.,* 2001). For the last many years, efforts are being made to develop an effective vaccine against HIV. But no significant achievement is on record. Only chemical solution for controlling this dreadful disease is not adequate. To control HIV (Human immunodeficiency virus) and other diseases, plant-based (PB) vaccines could fulfil many of the requirements due to their low cost and high efficacy (Plasson *et al.,* 2009; Pogue *et al.,* 2010). Since the inception of the 21st century, different plants have been reported with an expression of HIV-1 antigens. For instance, the *PA* expression in transgenic *Nicotiana* sp. having LF (lethal factor) was demonstrated and concluded positive control measure (Aziz *et al.,* 2005). Similarly, *PA* from transgenic tomatoes caused immunogenicity in mice (Aziz *et al.,* 2005) that verified HIV-1 subtype G protein production in tobacco plants. This protein caused humoral immune responses in mice already injected with an HIV-DNA vaccine. This result displays a positive correlation between protein and anti-viral responses. Other than HIV, Pag gene express a protein (anthrax protective antigen-PA) in tobacco that is used against anthrax (Meyers *et al.*, 2008). Moreover, *the human papillomavirus* vaccine was released many years ago. Additionally, antigens, vaccines from eatables would be cheaper and easily storable. So, these would be in access of all. To address related issues and concerns, scientists are thinking of processing edible plants into different forms, thus making more viable and consistent options. The dilemma is that the developing and underdeveloped countries have a low capacity to adopt high-cost vaccines. To make a substitute for high-cost vaccines, engineered plants are the paramount option in hand as the preferred bio-systems. Scientific progress in this field has led to the growth of delicate systems offered for the production of plant-based recombinant pharmaceuticals. Numerous plant species are currently acquiescent to genetic exploitation (Zaynab *et al.,* 2017; Hussain *et al.,* 2018; Noman *et al.,* 2018).

Making "edible vaccines and antibodies" in Plants

For fighting diseases, high-efficiency vaccines are prospective hope for human beings. However, due to the lofty prices of classical vaccines, people in many developing countries cannot use them. Every year, a large number of deaths are caused by infectious diseases (Sym *et al.,* 2009). New pathogen growth and diseases such as HIV (Human Immunodeficiency Virus), HCV (Hepatitis C Virus) have resulted in hue and cry across the globe. Despite efforts at all levels, the situation is getting worst day by day. Being cost effective and easy way, different vaccines are being used against infectious as well as noninfectious diseases (Table 3: Pogue *et al.,* 2010).

The edible vaccine causes an immune response after oral administration. These vaccines are the incorporation of antigenic material into an edible plant part. Although this is an auspicious substitute, still its commercialization is facing issues in different countries (Guan *et al.,* 2013). A plant expression system for production of edible protein is employed to express a particular antigenic peptide. So far, different plant species have been used for the production of edible vaccines. For example, transgenic banana, rice, tobacco and tomato plants have been used for edible vaccine production. Transgenic rice is the most extensively used plant for this purpose. Some scientists consider potato as an ideal plant expression system for the production of edible proteins. Following data (Table 4) describes different plant expression system for edible vaccine production.

Over the years, utilization of whole plants for recombinant proteins synthesis has shown economic benefits and safety advantages in comparison microbial as well as mammalian expression systems (Rybicki, 2018). But, whole plants based recombinant proteins production systems are devoid of many intrinsic advantages of cultured cells such as control over growth conditions, high

Table 3: Different plant expression systems with transformed genes have successfully yeilded different vaccines against various diseases

Disease	Genes used	Expression system	Results	References
Allergy	Cry j I & Cry j II	*Oryza sativa*	Immunot herapy against allergy	Takagi *et al.* (2005)
Diarrheal diseases	Enterotoxigenic *E. coli* and norovirus	*Solanum tuberosum*	LT-B induce approximately same mucosa lB-cell priming as takes place after enterotoxigenic *E. coli* attack	Tacket (2007)
Diphtheria, Pertussis, Tetanus	DPT	*Lycopersicum esculentum*	IgA and IgG antibodies in digestive system	Soria-Guerra *et al.* (2007)
Endemic disease in Africa Rinderpest Virus)	Hemagglutinin protein	*Pisum sativum*	H gene integration into the genome and protein expression level confirmed	Satyavathi *et al.* (2003)
Epidemic acute gastroenteritis	Norwalk Virus Capsid Protein	*Nicotiana tabbacum*	Plant derived rNV was orally active	Mason *et al.* (1996)
Foot and mouth disease	P1 gene	*Oryza sativa*	Improved immunisation	Wang *et al.* (2012)
Hepatitis B	HbsAg	*Musa sp.*	High HBsAg expression level recorded in leaves	Kumar *et al.* (2005)
	Hepatitis B surface antigen HBsAg)	*Solanum tuberosum*	transformed Antigen generated systemic response in the human intestine	Thanavala *et al.* (2005)
	HbsAg		transformed Antigen generated systemic response in the human intestine	Hayden *et al.* (2012)
Procrine Reproductive and Respiratory Syndrom	PRRS virus matrix protein	*Zea mays*	Cellular immune response	Hu *et al.* (2012)
Psittacosis	MOMP	*Oryza sativa*	activated Humoral and cell mediated immunity	Zhang *et al.* (2013)
Rotaviral diarrhea	eBRV4	*Medicago trucatula*	It induced Lactogenic immunity	Wigdorovitz *et al.* (2004)
Swine edema disease	Vt2e-B & FedA	*Nicotiana tabbacum*	Function against infection like verocytotoxic *E. coli*	Rossi *et al.* (2013)

This table elaborates genes that have been transformed in different plants for production of chemicals to be used against human diseases

Table 4: Comparative attributes statement for different recombinant protein expression systems reveals their efficiency and other characteristics for treatment of human deiseases (Ma *et al*., 2003)

	Swiftness	Scalability	BioSafety	Folding accuracy	Multimeric assembly	Glycosylation issues	Capital investment	Operating Cost	Storage	Product Homogeneity	Yield	Ethical concerns
Mammalian cell culture	√√	√	√√	√√√√√	√√√	√√√√	√	√√	N_2	High	Medium to high	Medium
Bacterial cell	√√√√√	√√	√√√	√	√	√	√√√	√√√√	-20°C	Low	Medium	Medium
Yeast cells	√√√√	√√√	√√√√	√√	√√	√√	√√	√√√	-20°C	Medium	High	Medium
Transgenic plants	√√√	√√√√√	√√√√√	√√√	√√√√	√√√	√√√√√	√√√√√	Room temp.	Medium to High	High	Medium
Transgenic animals	√	√√√√	√	√√√√	√√√√√	√√√√√	√√√√	√	N_2	Low	High	High

√√√√√= *Best of attribute*
√= Minimum of described attribute

containment level *etc*. (Hellwig *et al.,* 2004; Noman *et al.,* 2019). Seminal contributions have been made by plant cell cultures in the form of combined merits of whole-plant systems with microbial and animal cell cultures. Most early studies, as well as current work, focus on the production of important pharmacological metabolites (Reski *et al.*, 2015; Babura *et al.,* 2017; Yoo *et al.,* 2017). Consistent with the literature, this research reveals the fact that not much recombinant proteins have been produced at a commercial scale by employing plant cell cultures. But, several research groups have targetted the commercial feasibility of these production systems.

Concept of bio-pharming has aided generously in the development of new vaccines and antibodies (Walmsley and Arntzen, 2003). Plant-derived antibodies are considered particularly appropriate for relevant immunotherapy. A quarter of researchers argued that edible plant parts, *i.e*., banana could be exploited as bio-factories for vaccine production (Paul *et al.,* 2017). But it is thought that by adopting this way, we could get needle-free vaccines and that will not require other chemicals for stimulating immune reply. Obtained needleless vaccines can be directly used as fruits, vegetables or in other forms (Fig. 2).

One of the major benefits of these edible vaccines is the release of antigens in intestines and further transport to a circulatory system. Therefore, it is agreed that edible vaccines can excite mucosal as well as systemic immunity leading to privileged protection in comparison with routine vaccines. With proven efficacy and performance, transgenic plants based vaccines are continually increasing, *e.g., Norwalk* virus capsid protein. For the production of diverse antigens, plant transgenic vectors were utilized in plants for induction of vaccine production for the disease treatment (Noman *et al*., 2017b; Rigano *et al.*, 2009; Tiwari *et al*., 2009). Hiatt *et al.* (1989) firstly presented plantibodies expressions (transgenic plants antibodies). The plant-based vaccines production in transgenic tobacco (protein from Streptococci) was first reported in 1990 (Mason *et al.,* 1992). Later, plants were designated as bioreactors and a broad range of vaccines was produced. The transgenic plants derived vaccines proved to be highly efficient for immunization against bacterial or viral infections. Many of such vaccines have been released (Ko and Koprowski, 2005; Ma *et al.,* 2005), while many are

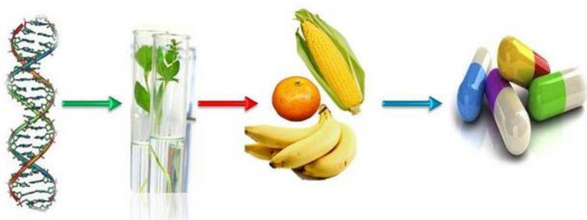

Fig. 2: How biotechnology is working for plant based drug production? Biotechnology manipulates different genes and proteinds of plant origin to generate products of medicinal significance. Genes for specific proteins are spliced into host DNA. Transgenic plants are regenerated that express spliced protein. From these plants different products like fruits or seeds are harvested and processed into specialized forms as consumable.

in clinical trials. Being edible, the antigens derived from engineered plants are gaining high market value every day. Many vaccines, enzymes and multiple proteins having pharmaceutical importance have been detected and isolated from plants. Though vigorous recombinant proteins have been reported, yet the problem is low product yield and quality (Paul and Ma, 2011; Linnhoff *et al.*, 2017; Paul *et al.*, 2017). This is somewhat counterintuitive. The most striking output emerged from the data is that antibodies in plants can be expressed with total soluble protein (TSP) yields up to 8%. Conversely, recombinant antigen production in plants is very low and hardly goes beyond 0.4% of TSP (Warzecha and Mason, 2003). Weak expression, wrong targeting and rapid degradation ultimately result in low yield.

Despite some limitations, plants are still very suitable for the production of pharmaceutical proteins. The relevance of plants as biopharming units is supported by the use of plants such as legumes, cereals and algae to generate phytoceuticals and biopharmaceutical proteins (Twyman *et al.*, 2003; Paul and Ma, 2011). Likewise, potato is regarded as a model plant for oral vaccines production (Polkinghorne *et al.*, 2005). Over the last few years, researchers have used tomato as an expression system. Antigen genes for various infectious diseases like hepatitis, AIDS and rabies have been successfully integrated into a tomato plant. Additionally, cultured carrot cell proplastids have expressed recombinant proteins (Daniell *et al.*, 2001) and that carrot is credited with the preservation of target protein's structural integrity (Muller *et al.*, 2003). Among the fruit plants, foreign proteins expression through promoter MaExp1 in *Musa paradisiaca* had been demonstrated (Trivedi and Nath, 2004; Paul *et al.*, 2017). Papaya had also been investigated to produce vaccines (Carter III and Langridge, 2002). All of these examples support this strategy for vaccine production. The insights from the presented data assist in understanding the success of biotechnological application upon plants for the production of phytoceuticals. Now, need is to expand this on a large scale for assessment of possibilities of plant systemic and oral immunization in coming years (Noman *et al.*, 2016; Linnhoff *et al.*, 2017; Pniewski *et al.*, 2017).

Traditional as well as some non-traditional crop plants have been considered very useful as a PB protein production system. Scientists have agreed upon using Moss and Lemna sp. as one of the adaptable PMP (plant-made pharmaceuticals) production system (Lang *et al.*, 2005; Stomp, 2005; Reski *et al.*, 2015). Moss undergoes HR at nuclear loci at a momentous rate (Lang *et al.*, 2005), that allows researchers to architect transgenes for integration into precise regions in moss genome. A more systematic and theoretical strategy will be beneficial in decreasing unevenness among transgenic cultivars. Additionally, we think that strong knowledge about transgene integration site can be beneficial for regulatory approval and application for IP protection. Therefore, we are in a position to infer that HR based moss genome targeted mutation can be adopted for the production of new lines with enhanced protein engineering and processing, *e.g.*, engineering of glycosylation pattern (Koprivova *et al.*, 2004).

Many plants, such as *Medicago sativa* produce plenty of soluble proteins and can be highly valuable for the production of oral vaccines (Stoger *et al.*, 2005). Cereals produce more proteins and less secondary metabolites that make them effectual bioreactors for recombinant proteins. For instance, maize was studied for different recombinant antibodies. Due to a correlation between endospermic proteins and antigen concentration (Ahmad *et al.*, 2012), *Oryza sativa* has been focused for protein expression by using endosperm-specific promoters (Hood *et al.*, 2002; Nicholson *et al.*, 2005). Not only for human use but also animals, these transgenic PB vaccines have been trialed for prevention of infections. Different successful responses like serum production, mucosal antibodies and elevating cytokine levels have been noted in animals after application of transgenic plant vaccines. Still, more considerable efforts are needed to ensure the accessibility and applicability of transgenic plant products for animals and humans.

Safety, Commercialization and Risks Related to Phytoceutical Producing Transgenic Plants

The existing awareness about phytoceuticals and medicinal plants can help in combating the shortages of commercial medicines and as a choice for developing therapeutics (Paul *et al.*, 2017). PB pharmaceuticals acquire several advantages over other techniques (Linnhoff *et al.*, 2017) like mammalian cell culture methods *etc*. The available literature supports low cost as one of the prime advantages. In addition to being quite safe for humans and environment, massive production capability is another advantage. For this, seed development is needed and more area can be brought under cultivation. Based on existing research findings, phytoceuticals are believed to be safer inherently than recombinant proteins obtained from microorganisms (Aziz *et al.*, 2005; Maughan *et al.*, 2018).

For transgenic plant production, many researchers believe that horticultural and agricultural plants can be exploited as biofactories for vaccine production (Babura *et al.,* 2017; Paul *et al.,* 2017; Rybicki, 2018). Prior studies that have noted the importance of that we can get needle-free vaccines and that will not require other chemicals for stimulating immune reply (Paul *et al.,* 2017). Clinical trials of these vaccines are going on and many technical objections have been removed. But indeed, some questions are unanswered. Before marketing, GM crops are critically evaluated for ensuring safety. Transgenic plants safety appraisal is an exciting research aspect for various disciplines like agronomy, ecology and molecular biology. Normally, the main focus relies on food and environmental safety (Chassy, 2010). During a review of GM plants, other risks are also taken into consideration predominantly for insect/pest or disease resistance/tolerance characteristics. Since the late 1980s, Coordinated Framework for Regulation of Biotechnology (CFRB) is being followed as an official policy which comprehensively describes SOP (standard operating procedure) for evaluation of products developed by using modern protocols. Additionally, keeping in view the advancement and adoption of GM products, NIH (National Institute of Health) has introduced strict regulations for disposal of GM plants. To date, other chief agencies like USDA, EPA and FDA have formulated statutes and guidelines for the assessment and release of GMOs. It is commonly agreed that all regulatory authorities must be entirely liable for ensuring the environment as well as human health safety in the presence of GM crops. A few years ago, many problems were highlighted before the commercial release of biotech crops. Many of the governments have imposed restrictions on GM crop cultivation. Southern Australia had suspended the cultivation of GM food crops, especially canola during 2006–2008 (Ahmad *et al.,* 2012). In 2009, the Indian government allowed the commercialization of *Bt* brinjal in the country, but the negative public response forced the govt. to review the decision and this crop faced moratorium. Some countries after debate have allowed cultivation of GM plants. For example, after many years of a prohibition, the commercial-scale farming of GM canola in New South Wales was approved in 2008 (Ahmad *et al.,* 2012).

Although obtaining drug products from different crop and non-crop plants is very promising, yet it carries many risks. In particular, the use of food crops for this purpose has faced criticism. The major fraction of the public is positive regarding the capability of biotechnology to improve our life quality and standard. However, there are noticeable differences between overall support when aims and objectives are related to medical biotechnology. For example, a major proportion of PB dietary proteins are digested and absorbed by the body with no adverse effects. But some other proteins can be toxic or possess anti-nutrient activity, *e.g*., trypsin inhibitors (Delaney *et al.,* 2008). There have been numerous studies representing many known noxious or allergenic proteins. By using this kind of information, it is probable to check if a recently introduced protein resembles other proteins, whether toxic or allergenic (Goodman *et al.,* 2008).

Whereas, transgenic pharma plants may cause threats to beneficial insects that feed upon their pollens and to wildlife that eat GM and other engineered plants. This may be due to the production of a considerable amount of drugs and chemicals (Ahmad *et al.,* 2012). This issue is leaving question marks on the safety of phytoceutical yielding transgenics. Due to the confusion in the consumer mind, commercialization of products has been affected. Moreover, the availability of patents for specific plants and technologies is also hindering the progress in the safety of transgenic pharma plants. A series of recent research have designated, *Zea mays* as popular biopharma crop.

In the same way, some non-food plants like soybean or tobacco are also being used. For tobacco, the major problem is, it should be processed green as we may encounter protein change or deterioration in case of drying. This thing can hamper the desired productivity of plants. At commercial scale production, gene-containment actions, *e.g*., chloroplast transformation and male sterility are not adequately secured and chances of leak genetic material are evident. According to the critics, transferring new genes in human food is in fact alteration in the chemistry of food which can activate the body to respond in a different way to that food, thereby may cause allergies or toxicity. Moreover, more than a few transgenic crops harbor genes for antibiotic-resistance that might be taken up by bacteria and lead to increased resistance against antibiotics. Many wild plants with strong medicinal potential are disappearing due to anthropogenic pressures. Additionally, habitats of some species need immediate protection. On the other hand, researchers should pay attention to field evaluation of transgenic plants as people still think that medicinally valuable transgenics are limited to labs.

As we discussed earlier, there is a steady boost in the employment of GM organisms for food or vital produce (Ahmad *et al.,* 2012; Linnhoff *et al.,* 2017). To ensure environmental safety, we can use a green resource to make drugs quicker, cheaper and safer for a human being. This would be a highly fruitful area for further work. The plant biotechnologists must keep in mind that the developed and developing transformants are safe enough. It is their professional liability to ensure that this research is only applied for human welfare and not to cause destruction.

Conclusions and Future Perspectives

Introduction of GE and related tools has facilitated plant scientists for production of various PB products like biofuels, nutraceuticals and off course drug development. Despite controversies on transgenic crops, many authors have recognized considerable economic rewards from agri-biotechnology. Achievements of plant scientists and biotechnologists are not hidden as GM plants are being widely used for the making phytoceuticals. This document has thrown up many questions in need of further investigation. As we have reviewed possible results of PB vaccines, but some problems like dosage, mode of administration require intensive research. For medicine purpose, GM plants need to be strictly evaluated for safety. Other than an apparent success story, need is to alleviate different concerns for giving a real boom to phytoceuticals. Therefore, One of the tough challenges for all researchers in this domain is the efficient utilization of phytoceuticals along with large scale studies. Recent history reveals several benefits from transgenic PB edible vaccines by induction of immunization against bacterial and viral diseases. Undoubtedly, edible vaccines are highly secure plus cost-effective. Conversely, critics are still claiming that transgenic PB products are harmful to humans and the environment. The question that then naturally arises is toxicity from altered product composition due to GE. As the authors note earlier, every state is required to formulate specific laws for GM plants utilization. So this is the real time to focus non-crop plants as well for management and development as the source of a natural and recombinant drug. The facts reviewed sturdily favoring that transgenic plant expression systems are ahead of the proof of concept step. These propose a lawful cost-competitive substitute for the production of recombinant proteins and other medicines. Transgenic PB medicinal products development symbolize a multifaceted endeavor requiring an incredibly integrated multidisciplinary advance. Undoubtedly, the topical biotechnological progress, scientific advances and research drifts point out that biotechnological medicinal product will be amid the chief resources of novel drugs in future.

References

Ahmad, P., M. Ashraf, M. Younis, X. Hu, A. Kumar, N.A. Akram and F. Al-Qurainy, 2012. Role of transgenic plants in agriculture and biopharming. *Biotechnol. Adv.*, 30: 524–540

Aluru, M., Y. Xu, R. Guo, Z. Wang, S. Li, W. White, K. Wang and S. Rodermel, 2008. Generation of transgenic maize with enhanced provitamin A content. *J. Exp. Bot.*, 59: 3551–3562

Anai, T., M. Koga, H. Tanaka, T. Kinoshita, S. Rahman and Y. Takagi, 2003. Improvement of rice (*Oryza sativa* L.) seed oil quality through introduction of a soybean microsomal omega-3 fatty acid desaturase gene. *Plant Cell Rep.*, 21: 988–992

Arakawa, T., D.K.X. Chong and W.H.R. Langridge, 1998. Efficacy of a food plant-based oral cholera toxin B subunit vaccine. *Nat. Biotechnol.*, 16: 292–297

Ashraf, M. and N.A. Akram, 2009. Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison. *Biotechnol. Adv.*, 27: 744–752

Aziz, M.A., D. Sikriwal, S. Singh, S. Jarugula, P.A. Kumar and R. Bhatnagar, 2005. Transformation of an edible crop with the *pagA* gene of *Bacillus anthracis*. *FASEB J.*, 19: 1501–1503

Babura, S.R., S.N.A. Abdullah and H. Khaza, 2017. Advances in genetic improvement for tocotrienol production: A review. *J. Nutr. Sci. Vitaminol.*, 63: 215–221

Bagchi, D., 2006. *Nutraceuticals and Functional Foods Regulations in the United States and Around the World*. Elsevier, Amsterdam, The Netherlands

Batabyal, A.A., Y. Higano and P. Nijkamp, 2019. *Disease, Human Health, and Regional Growth and Development in Asia*. Springer, Dodreschst, The Netherlands

Bulley, S., M. Wright, C. Rommens, H. Yan, M. Rassam, K. Lin-Wang, C. Andre, D. Brewster, S. Karunairetnam and A.C. Allan, 2012. Enhancing ascorbate in fruits and tubers through over-expression of the l-galactose pathway gene GDP-l-galactose phosphorylase. *Plant Biotechnol. J.*, 10: 390–397

Burgess, C., M. O'Connell-Motherway, W. Sybesma, J. Hugenholtz and D.V. Sinderen, 2004. Riboflavin production in *Lactococcus lactis*: potential for in situ production of vitamin-enriched foods. *Appl. Environ. Microbiol.*, 70: 5769–5777

Cahoon, E.B., S.E. Hall, K.G. Ripp, T.S. Ganzke, W.D. Hitz and S.J. Coughlan, 2003. Metabolic redesign of vitamin E biosynthesis in plants for tocotrienol production and increased antioxidant content. *Nat. Biotechnol.*, 21: 1082–1087

Caimi, P.G., L.M. McCole, T.M. Klein and P.S. Kerr, 1996. Fructan accumulation and sucrose metabolism in transgenic maize endosperm expressing a *Bacillus amyloliquefaciens* SacB gene. *Plant Physiol.*, 110: 355–363

Carter III, J.E. and W.H.R. Langridge, 2002. Plant-based vaccines for protection against infectious and autoimmune diseases. *Crit. Rev. Plant Sci.*, 21: 93–109

Chakraborty, S., N. Chakraborty and A. Datta, 2000. Increased nutritive value of transgenic potato by expressing a nonallergenic seed albumin gene from *Amaranthus hypochondriacus*. *Proc. Natl. Acad. Sci.*, 97: 3724–3729

Chassy, B.M., 2010. Food safety risks and consumer health. *New Biotechnol.*, 27: 534–544

Chen, Z., T.E. Young, J. Ling, S.C. Chang and D.R. Gallie, 2003. Increasing vitamin C content of plants through enhanced ascorbate recycling. *Proc. Natl. Acad. Sci.*, 100: 3525–3530

Chikwamba, R.K., M.P. Scott, L.B. Mejía, H.S. Mason and K. Wang, 2003. Localization of a bacterial protein in starch granules of transgenic maize kernels. *Proc. Natl. Acad. Sci.*, 100: 11127–11132

Cong, L., C. Wang, L. Chen, H. Liu, G. Yang and G. He, 2009. Expression of phytoene synthase1 and carotene desaturase *CrtI* genes result in an increase in the total carotenoids content in transgenic elite wheat (*Triticum aestivum* L.). *J. Agric. Food Chem.*, 57: 8652–8660

Daniell, H., S.J. Streatfield and K. Wycoff, 2001. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci.*, 6: 219–226

Delaney, B., J.D. Astwood, H. Cunny, R.E. Conn, C. Herouet-Guicheney, S. MacIntosh, L.S. Meyer, L. Privalle, Y. Gao and J. Mattsson, 2008. Evaluation of protein safety in the context of agricultural biotechnology. *Food Chem. Toxicol.*, 46: 71–97

Diretto, G., S. Al-Babili, R. Tavazza, V. Papacchioli, P. Beyer and G. Giuliano, 2007. Metabolic engineering of potato carotenoid content through tuber-specific overexpression of a bacterial mini-pathway. *PLoS One*, 2: 1–8

Drakakaki, G., S. Marcel, R.P. Glahn, E.K. Lund, S. Pariagh, R. Fischer, P. Christou and E. Stoger, 2005. Endosperm-specific co-expression of recombinant soybean ferritin and *Aspergillus* phytase in maize results in significant increases in the levels of bioavailable iron. *Plant Mol. Biol.*, 59: 869–880

Elbehri, A., 2005. Biopharming and the food system: examining the potential benefits and risks. *AgBioForum*, 8: 18–25

Elkholy, S.F., R.M. Ismail, A. Bahieldin, A.S. Sadik and M.A. Madkour, 2009. Expression of hepatitis B surface antigen (HBsAg) gene in transgenic banana (*Musa* spp.). *Arab. J. Biotechnol.*, 12: 291–302

Fischer, R., E. Stoger, S. Schillberg, P. Christou and R.M. Twyman, 2004. Plant-based production of biopharmaceuticals. *Curr. Opin. Plant Biol.*, 7: 152–158

Galili, G., S. Galili, E. Lewinsohn and Y. Tadmor, 2002. Genetic, molecular, and genomic approaches to improve the value of plant foods and feeds. *Crit. Rev. Plant Sci.*, 21: 167–204

Goodman, R.E., S. Vieths, H.A. Sampson, D. Hill, M. Ebisawa, S.L. Taylor and R.V. Ree, 2008. Allergenicity assessment of genetically modified crops—what makes sense? *Nat. Biotechnol.*, 26: 73–81

Goto, F., T. Yoshihara and H. Saiki, 2000. Iron accumulation and enhanced growth in transgenic lettuce plants expressing the iron-binding protein ferritin. *Theor. Appl. Genet.*, 100: 658–664

Grusak, M.A., 2005. Golden Rice gets a boost from maize. *Nat. Biotechnol.*, 23: 429

Guan, Z.J., B. Guo, Y.L. Huo, Z.P. Guan, J.K. Dai and Y.H. Wei, 2013. Recent advances and safety issues of transgenic plant-derived vaccines. *Appl. Microbiol. Biotechnol.*, 97: 2817–2840

Hayden, C.A., S.J. Streatfield, B.J. Lamphear, G.M. Fake, T.K. Keener, J.H. Walker, J.D. Clements, D.D. Turner, I.R. Tizard and J.A. Howard, 2012. Bioencapsulation of the hepatitis B surface antigen and its use as an effective oral immunogen. *Vaccine*, 30: 2937–2942

Hellwege, E.M., S. Czapla, A. Jahnke, L. Willmitzer and A.G. Heyer, 2000. Transgenic potato (*Solanum tuberosum*) tubers synthesize the full spectrum of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) roots. *Proc. Natl. Acad. Sci.*, 97: 8699–8704

Hellwege, E.M., D. Gritscher, L. Willmitzer and A.G. Heyer, 1997. Transgenic potato tubers accumulate high levels of 1-kestose and nystose: functional identification of a sucrose sucrose 1-fructosyltransferase of artichoke (*Cynara scolymus*) blossom discs. *Plant J.*, 12: 1057–1065

Hellwig, S., J. Drossard, R.M. Twyman and R. Fischer, 2004. Plant cell cultures for the production of recombinant proteins. *Nat. Biotechnol.*, 22: 1415–1423

Hiatt, A., R. Caffferkey and K. Bowdish, 1989. Production of antibodies in transgenic plants. *Nature*, 342: 76–78

Hood, E.E., S.L. Woodard and M.E. Horn, 2002. Monoclonal antibody manufacturing in transgenic plants myths and realities. *Curr. Opin. Biotechnol.*, 13: 630–635

Hu, J., Y. Ni, B.A. Dryman, X. Meng and C. Zhang, 2012. Immunogenicity study of plant-made oral subunit vaccine against porcine reproductive and respiratory syndrome virus (PRRSV). *Vaccine*, 30: 2068–2074

Huang, Z., I. Dry, D. Webster, R. Strugnell and S. Wesselingh, 2001. Plant-derived measles virus hemagglutinin protein induces neutralizing antibodies in mice. *Vaccine*, 19: 2163–2171

Hussain, A., X. Li, Y. Weng, Z. Liu, M.F. Ashraf, A. Noman, S. Yang, M. Ifnan, S. Qiu, Y. Yang, D. Guan and S. He, 2018. CaWRKY22 Acts as a Positive Regulator in Pepper Response to *Ralstonia solanacearum* by Constituting Networks with CaWRKY6, CaWRKY27, CaWRKY40 and CaWRKY58. *Intl. J. Mol. Sci.*, 19: 1426–1445

Islam, W., A. Noman, M. Qasim and L. Wang, 2018. Plant responses to pathogen attack: small rnas in focus. *Intl. J. Mol. Sci.*, 19: 515–538

Joh, L.D. and J.S. VanderGheynst, 2006. Agroinfiltration of plant tissues for production of high-value recombinant proteins: an alternative to production in transgenic crops. *J. Sci. Food Agric.*, 86: 2002–2004

Johnson, A.A.T., B. Kyriacou, D.L. Callahan, L. Carruthers, J. Stangoulis, E. Lombi and M. Tester, 2011. Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. *PloS one*, 6: 1–11

Kapusta, J., A. Modelska, M. Figlerowicz, T. Pniewski, M. Letellier, O. Lisowa, V. Yusibov, H. Koprowski, A. Plucienniczak and A.B. Legocki, 1999. A plant-derived edible vaccine against hepatitis B virus. *FASEB J.*, 13: 1796–1799

Katsube, T., N. Kurisaka, M. Ogawa, N. Maruyama, R. Ohtsuka, S. Utsumi and F. Takaiwa, 1999. Accumulation of soybean glycinin and its assembly with the glutelins in rice. *Plant Physiol.*, 120: 1063–1074

Khan, M.I., Y. Zhang, Z. Liu, J. Hu, C. Liu, S. Yang, A. Hussain, M.F. Ashraf, A. Noman, L. Shen, X. Xia, F. Yang, D. Guan and S. He, 2018. CaWRKY40b in Pepper Acts as a Negative Regulator in Response to *Ralstonia solanacearum* by Directly Modulating Defense Genes Including CaWRKY40. *Intl. J. Mol. Sci.*, 19: 1403

Ko, K. and H. Koprowski, 2005. Plant biopharming of monoclonal antibodies. *Vir. Res.*, 111: 93–100

Koprivova, A., C. Stemmer, F. Altmann, A. Hoffmann, S. Kopriva, G. Gorr, R. Reski and E.L. Decker, 2004. Targeted knockouts of Physcomitrella lacking plant-specific immunogenic N-glycans. *Plant Biotechnol. J.*, 2: 517–523

Kumar, B.V., T. Raja, M. Wani, S. Sheikh, M. Lone, G. Nabi, M. Azooz, M. Younis, M. Sarwat and P. Ahmad, 2013. Transgenic plants as green factories for vaccine production. *Afr. J. Biotechnol.*, 12: 6147–6158

Kumar, G.S., T. Ganapathi, C. Revathi, L. Srinivas and V. Bapat, 2005. Expression of hepatitis B surface antigen in transgenic banana plants. *Planta*, 222: 484–493

Lai, J. and J. Messing, 2002. Increasing maize seed methionine by mRNA stability. *Plant J.*, 30: 395–402

Lang, D., J. Eisinger, R. Reski and S. Rensing, 2005. Representation and High-Quality Annotation of the *Physcomitrella patens* Transcriptome Demonstrates a High Proportion of Proteins Involved in Metabolism in Mosses. *Plant Biol.*, 7: 238–250

Lau, J.M. and S.S. Korban, 2010. Transgenic apple expressing an antigenic protein of the human respiratory syncytial virus. *J. Plant Physiol.*, 167: 920–927

LeDuc, D.L., A.S. Tarun, M. Montes-Bayon, J. Meija, M.F. Malit, C.P. Wu, M. AbdelSamie, C.Y. Chiang, A. Tagmount and M. deSouza, 2004. Overexpression of selenocysteine methyltransferase in *Arabidopsis* and Indian mustard increases selenium tolerance and accumulation. *Plant Physiol.*, 135: 377–383

Lee, S., D.P. Persson, T.H. Hansen, S. Husted, J.K. Schjoerring, Y.S. Kim, U.S. Jeon, Y.K. Kim, Y. Kakei and H. Masuda, 2011. Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase. *Plant Biotechnol. J.*, 9: 865–873

Linnhoff, S., E. Volovich, H.M. Martin and L.M. Smith, 2017. An examination of millennials' attitudes toward genetically modified organism (GMO) foods: is it Franken-food or super-food? *Intl. J. Agric. Resour. Govern. Ecol.*, 13: 371–390

Liu, Q., S.P. Singh and A.G. Green, 2002. High-stearic and high-oleic cottonseed oils produced by hairpin RNA-mediated post-transcriptional gene silencing. *Plant Physiol.*, 129: 1732–1743

Liu, Z., L. Shi, S. Yang, Y. Lin, Y. Weng, X. Li, A. Hussain, A. Noman and S. He, 2017. Functional and promoter analysis of CHIIV3, a chitinase of pepper plant, in response to phytophthora capsici infection. *Intl. J. Mol. Sci.*, 18: 1661

Lopez, A.B., J.V. Eck, B.J. Conlin, D.J. Paolillo, J. O'neill and L. Li, 2008. Effect of the cauliflower Or transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *J. Exp. Bot.*, 59: 213–223

Lukaszewicz, M., I. Matysiak-Kata, J. Skala, I. Fecka, W. Cisowski and J. Szopa, 2004. Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic compounds content. *J. Agric. Food Chem.*, 52: 1526–1533

Ma, J.K., P.M. Drake, D. Chargelegue, P. Obregon and A. Prada, 2005. Antibody processing and engineering in plants, and new strategies for vaccine production. *Vaccine*, 23: 1814–1818

Mason, H.S., J.M. Ball, J.J. Shi, X. Jiang, M.K. Estes and C.J. Arntzen, 1996. Expression of Norwalk virus capsid protein in transgenic tobacco and potato and its oral immunogenicity in mice. *Proc. Natl. Acad. Sci.*, 93: 5335–5340

Mason, H.S., D. Lam and C.J. Arntzen, 1992. Expression of hepatitis B surface antigen in transgenic plants. *Proc. Natl. Acad. Sci.*, 89: 11745–11749

Matsumoto, Y., S. Suzuki, T. Nozoye, T. Yamakawa, Y. Takashima, T. Arakawa, N. Tsuji, F. Takaiwa and Y. Hayashi, 2009. Oral immunogenicity and protective efficacy in mice of transgenic rice plants producing a vaccine candidate antigen (As16) of *Ascaris suum* fused with cholera toxin B subunit. *Trans. Res.*, 18: 185–193

Maughan, R.J., L.M. Burke, J. Dvorak, D.E. Larson-Meyer, P. Peeling, S.M. Phillips, E.S. Rawson, N.P. Walsh, I. Garthe, H. Geyer, R. Meeusen, L.V. Loon, S.M. Shirreffs, L.L. Spriet, M. Stuart, A. Vernec, K. Currell, V.M. Ali, R.G.M. Budgett, A. Ljungqvist, M. Mountjoy, Y. Pitsiladis, T. Soligard, U. Erdener and L. Engebretsen, 2018. IOC Consensus Statement: Dietary Supplements and the High-Performance Athlete. *Intl. J. Sport Nutr. Exer. Metabol.*, 28: 104–125

Meyers, A., E. Chakauya, E. Shephard, F.L. Tanzer, J. Maclean, A. Lynch, A.L. Williamson and E.P. Rybicki, 2008. Expression of HIV-1 antigens in plants as potential subunit vaccines. *BMC Biotechnol.*, 8: 53

Mikschofsky, H., H. Schirrmeier, G.M. Keil, B. Lange, P.L. Polowick, W. Keller and I. Broer, 2009. Pea derived vaccines demonstrate high immunogenicity and protection in rabbits against rabbit haemorrhagic disease virus. *Plant Biotechnol. J.*, 7: 537–549

Muller, C.P., F. Fack, B. Damien and F.B. Bouche, 2003. Immunogenic measles antigens expressed in plants: role as an edible vaccine for adults. *Vaccine*, 21: 816–819

Naqvi, S., C. Zhu, G. Farre, K. Ramessar, L. Bassie, J. Breitenbach, D.P. Conesa, G. Ros, G. Sandmann and T. Capell, 2009. Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. *Proc. Natl. Acad. Sci.*, 106: 7762–7767

Nicholson, L., P. Gonzalez-Melendi, C.V. Dolleweerd, H. Tuck, Y. Perrin, J.K.C. Ma, R. Fischer, P. Christou and E. Stoger, 2005. A recombinant multimeric immunoglobulin expressed in rice shows assembly-dependent subcellular localization in endosperm cells. *Plant Biotechnol. J.*, 3: 115–127

Noman, A., M. Aqeel, N. Khalid, W. Islam, T. Sanaullah, M. Anwar, S. Khan, W. Ye and Y. Lou, 2019. Zinc finger protein transcription factors: Integrated line of action for plant antimicrobial activity. *Microb. Pathog.*, 132: 141–149

Noman, A., Z. Liu, S. Yang, L. Shen, A. Hussain, M.F. Ashraf, M.I. Khan and S. He, 2018. Expression and functional evaluation of CaZNF830 during pepper response to *Ralstonia solanacearum* or high temperature and humidity. *Microb. Pathog.*, 118: 336–346

Noman, A., M. Aqeel, J. Deng, N. Khalid, T. Sanaullah and H. Shuilin, 2017a. Biotechnological advancements for improving floral attributes in ornamental plants. *Front. Plant Sci.*, 8: 1–15

Noman, A., Z. Liu, M. Aqeel, M. Zainab, M.I. Khan, A. Hussain, M.F. Ashraf, X. Li, Y. Weng and S. He, 2017b. Basic leucine zipper domain transcription factors: the vanguards in plant immunity. *Biotechnol. Lett.*, 39: 1779–1791

Noman, A., R. Bashir, M. Aqeel, S. Anwer, W. Iftikhar, M. Zainab, S. Zafar, S. Khan, W. Islam and M. Adnan, 2016. Success of transgenic cotton (*Gossypium hirsutum* L.): Fiction or reality? *Cogent Food Agric.*, 2: 1–13

Noman, A., I. Hussain, Q. Ali, M.A. Ashraf and M.Z. Haider, 2013. Ethnobotanical studies of potential wild medicinal plants of Ormara, Gawadar, Pakistan. *Emir. J. Food Agric.*, 25: 751–759

Paine, J.A., C.A. Shipton, S. Chaggar, R.M. Howells, M.J. Kennedy, G. Vernon, S.Y. Wright, E. Hinchliffe, J.L. Adams, A.L. Silverstone and R. Drake, 2005. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat. Biotechnol.*, 23: 482–492

Park, S., M.P. Elless, J. Park, A. Jenkins, W. Lim, E. Chambers IV and K.D. Hirschi, 2009. Sensory analysis of calcium-biofortified lettuce. *Plant Biotechnol. J.*, 7: 106–117

Park, S., T.S. Kang, C.K. Kim, J.S. Han, S. Kim, R.H. Smith, L.M. Pike and K.D. Hirschi, 2005. Genetic manipulation for enhancing calcium content in potato tuber. *J. Agric. Food Chem.*, 53: 5598–5603

Paul, J.Y., H. Khanna, J. Kleidon, P. Hoang, J. Geijskes, J. Daniells, E. Zaplin, Y. Rosenberg, A. James, B. Mlalazi, P. Deo, G. Arinaitwe, P. Namanya, D. Becker, J. Tindamanyire, W. Tushemereirwe, R. Harding and J. Dale, 2017. Golden bananas in the field: elevated fruit pro-vitamin A from the expression of a single banana transgene. *Plant Biotechnol. J.*, 15: 520–532

Paul, M. and J.K.C. Ma, 2011. Plant-made pharmaceuticals: Leading products and production platforms. *Biotechnol. Appl. Biochem.*, 58: 58–67

Peter, R.E., K.L. Yu, T.A. Marchant and P.M. Rosenblum, 1990. Direct neural regulation of the teleost adenohypophysis. *J. Exp. Zool.*, 256: 84–89

Plasson, C., R. Michel, D. Lienard, C. Saint-Jore-Dupas, C. Sourrouille, G.G.D. March and V. Gomord, 2009. *Production of Recombinant Proteins in Suspension–Cultured Plant Cells Recombinant Proteins From Plants*, pp: 145–161. Springer

Pniewski, T., M. Czyż, K. Wyrwa, P. Bociąg, P. Krajewski and J. Kapusta, 2017. Micropropagation of transgenic lettuce containing HBsAg as a method of mass-scale production of standardised plant material for biofarming purposes. *Plant Cell Rep.*, 36: 49–60

Pogue, G.P., F. Vojdani, K.E. Palmer, E. Hiatt, S. Hume, J. Phelps, L. Long, N. Bohorova, D. Kim and M. Pauly, 2010. Production of pharmaceutical-grade recombinant aprotinin and a monoclonal antibody product using plant-based transient expression systems. *Plant Biotechnol. J.*, 8: 638–654

Polkinghorne, I., D. Hamerli, P. Cowan and J. Duckworth, 2005. Plant-based immunocontraceptive control of wildlife-"potentials, limitations, and possums". *Vaccine*, 23: 1847–1850

Qadri, F., A.M. Svennerholm, A. Faruque and R.B. Sack, 2005. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clin. Microbiol. Rev.*, 18: 465–483

Rapp, W., 2002. Development of soybeans with improved amino acid composition. *In: 93rd AOCS Annual Meeting and Expo*, pp: 79–86. Compaign (IL): American Oil Chemical Society Press

Reski, R., J. Parsons and E.L. Decker, 2015. Moss-made pharmaceuticals: from bench to bedside. *Plant Biotechnol. J.*, 13: 1191–1198

Richter, L.J., Y. Thanavala, C.J. Arntzen and H.S. Mason, 2000. Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat. Biotechnol.*, 18: 1167–1171

Rigano, M.M., C. Manna, A. Giulini, A. Vitale and T. Cardi, 2009. Plants as biofactories for the production of subunit vaccines against bio-security-related bacteria and viruses. *Vaccine*, 27: 3463–3466

Rosati, C., R. Aquilani, S. Dharmapuri, P. Pallara, C. Marusic, R. Tavazza, F. Bouvier, B. Camara and G. Giuliano, 2000. Metabolic engineering of beta-carotene and lycopene content in tomato fruit. *Plant J.*, 24: 413–420

Rossi, L., A.D. Giancamillo, S. Reggi, C. Domeneghini, A. Baldi, V. Sala, V. Dell'Orto, A. Coddens, E. Cox and C. Fogher, 2013. Expression of verocytotoxic *Escherichia coli* antigens in tobacco seeds and evaluation of gut immunity after oral administration in mouse model. *J. Vet. Sci.*, 14: 263–270

Roy, S., A. Tyagi, S. Tiwari, A. Singh, S.V. Sawant, P.K. Singh and R. Tuli, 2010. Rabies glycoprotein fused with B subunit of cholera toxin expressed in tobacco plants folds into biologically active pentameric protein. *Protein Express. Purif.*, 70: 184–190

Rybicki, E., 2018. History and Promise of Plant-Made Vaccines for Animals. *In: Prospects of Plant-Based Vaccines in Veterinary Medicine*, pp*:* 1–22. MacDonald, J. (Ed.) Springer International Publishing, Cham, Switzerland

Sandhu, J.S., S.F. Krasnyanski, L.L. Domier, S.S. Korban, M.D. Osadjan and D.E. Buetow, 2000. Oral immunization of mice with transgenic tomato fruit expressing respiratory syncytial virus-F protein induces a systemic immune response. *Trans. Res.*, 9: 127–135

Satyavathi, V., V. Prasad, M. Shaila and L.G. Sita, 2003. Expression of hemagglutinin protein of Rinderpest virus in transgenic pigeon pea [*Cajanus cajan* (L.) Millsp.] plants. *Plant Cell Rep.*, 21: 651–658

Schijlen, E., C.R.D. Vos, H. Jonker, H.V.D. Broeck, J. Molthoff, A.V. Tunen, S. Martens and A. Bovy, 2006. Pathway engineering for healthy phytochemicals leading to the production of novel flavonoids in tomato fruit. *Plant Biotechnol. J.*, 4: 433–444

Shinwari, Z.K. and M. Qaiser, 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot.*, 43: 5–10

Smeekens, S., 1997. Engineering plant metabolism. *Trends Plant Sci.*, 2: 286–287

Soria-Guerra, R.E., S. Rosales-Mendoza, C. Márquez-Mercado, R. López-Revilla, R. Castillo-Collazo and Á.G. Alpuche-Solís, 2007. Transgenic tomatoes express an antigenic polypeptide containing epitopes of the diphtheria, pertussis and tetanus exotoxins, encoded by a synthetic gene. *Plant Cell Rep.*, 26: 961–968

Staub, J.M., B. Garcia, J. Graves, P.T.J. Hajdukiewicz, P. Hunter, N. Nehra, V. Paradkar, M. Schlittler, J.A. Carroll, L. Spatola, D. Ward, G. Ye and D.A. Russell, 2000. High-yield production of a human therapeutic protein in tobacco chloroplasts. *Nat. Biotechnol.*, 18: 333–338

Stoger, E., J.K. Ma, R. Fischer and P. Christou, 2005. Sowing the seeds of success: pharmaceutical proteins from plants. *Curr. Opin. Biotechnol.*, 16: 167–173

Stomp, A.M., 2005. The duckweeds: a valuable plant for biomanufacturing. *Biotechnol. Ann. Rev.*, 11: 69–99

Storozhenko, S., V.D. Brouwer, M. Volckaert, O. Navarrete, D. Blancquaert, G.F. Zhang, W. Lambert and D.V.D. Straeten, 2007. Folate fortification of rice by metabolic engineering. *Nat. Biotechnol.*, 25: 1277–1280

Sun, S.S.M., 2008. Application of agricultural biotechnology to improve food nutrition and healthcare products. *Asia Pac. J. Clin. Nutr.*, 17: 87–90

Svennerholm, A.M., 2011. From cholera to enterotoxigenic *Escherichia coli* (ETEC) vaccine development. *Ind. J. Med. Res.*, 133: 188–194

Sym, D., P.N. Patel and G.M. Ei-Chaar, 2009. Seasonal, avian, and novel H1N1 influenza: prevention and treatment modalities. *Ann. Pharmacother.*, 43: 2001–2011

Tacket, C.O., 2007. Plant-based vaccines against diarrheal diseases. *Trans. Amer. Clin. Climatol. Assoc.*, 118: 79–87

Tacket, C.O., M.F. Pasetti, R. Edelman, J.A. Howard and S. Streatfield, 2004. Immunogenicity of recombinant LT-B delivered orally to humans in transgenic corn. *Vaccine*, 22: 4385–4389

Tacket, C.O., H.S. Mason, G. Losonsky, M.K. Estes, M.M. Levine and C.J. Arntzen, 2000. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J. Infect. Dis.*, 182: 302–305

Takagi, H., T. Hiroi, L. Yang, Y. Tada, Y. Yuki, K. Takamura, R. Ishimitsu, H. Kawauchi, H. Kiyono and F. Takaiwa, 2005. A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses. *Proc. Natl. Acad. Sci.*, 102: 17525–17530

Thanavala, Y. and A.A. Lugade, 2010. Oral transgenic plant-based vaccine for hepatitis B. *Immunol. Res.*, 46: 4–11

Thanavala, Y., M. Mahoney, S. Pal, A. Scott, L. Richter, N. Natarajan, P. Goodwin, C.J. Arntzen and H.S. Mason, 2005. Immunogenicity in humans of an edible vaccine for hepatitis B. *Proc. Natl. Acad. Sci.*, 102: 3378–3382

Thanavala, Y., Y. Yang, P. Lyons, H. Mason and C. Arntzen, 1995. Immunogenicity of transgenic plant-derived hepatitis B surface antigen. *Proc. Natl. Acad. Sci.*, 92: 3358–3361

Tiwari, S., P.C. Verma, P.K. Singh and R. Tuli, 2009. Plants as bioreactors for the production of vaccine antigens. *Biotechnol. Adv.*, 27: 449–467

Tregoning, J., P. Maliga, G. Dougan and P.J. Nixon, 2004. New advances in the production of edible plant vaccines: chloroplast expression of a tetanus vaccine antigen, TetC. *Phytochemistry*, 65: 989–994

Trivedi, P.K. and P. Nath, 2004. MaExp1, an ethylene-induced expansin from ripening banana fruit. *Plant Sci.*, 167: 1351–1358

Twyman, R.M., E. Stoger, S. Schillberg, P. Christou and R. Fischer, 2003. Molecular farming in plants: host systems and expression technology. *Trends Biotechnol.*, 21: 570–578

Vemuri, R., R. Gundamaraju, M.D. Shastri, S.D. Shukla, K. Kalpurath, M. Ball, S. Tristram, E.M. Shankar, K. Ahuja and R. Eri, 2018. Gut microbial changes, interactions, and their implications on human lifecycle: An ageing perspective. *BioMed Res. Intl.*, 2018: 1–13

Walmsley, A.M. and C.J. Arntzen, 2003. Plant cell factories and mucosal vaccines. *Curr. Opin. Biotechnol.*, 14: 145–150

Wang, Y., Q. Shen, Y. Jiang, Y. Song, L. Fang, S. Xiao and H. Chen, 2012. Immunogenicity of foot-and-mouth disease virus structural polyprotein P1 expressed in transgenic rice. *J. Vir. Meth.*, 181: 12–17

Warzecha, H. and H.S. Mason, 2003. Benefits and risks of antibody and vaccine production in transgenic plants. *J. Plant Physiol.*, 160: 755–764

Webster, D., M. Thomas, Z. Huang and S. Wesselingh, 2005. The development of a plant-based vaccine for measles. *Vaccine*, 23: 1859–1865

White, C.L., L.M. Tabe, H. Dove, J. Hamblin, P. Young, N. Phillips, R. Taylor, S. Gulati, J. Ashes and T.J.V. Higgins, 2001. Increased efficiency of wool growth and live weight gain in Merino sheep fed transgenic lupin seed containing sunflower albumin. *J. Sci. Food Agric.*, 81: 147–154

Wigdorovitz, A., M. Mozgovoj, M.J.D. Santos, V. Parreno, C. Gomez, D.M. Perez-Filgueira, K.G. Trono, R.D. Ríos, P.M. Franzone and F. Fernandez, 2004. Protective lactogenic immunity conferred by an edible peptide vaccine to bovine rotavirus produced in transgenic plants. *J. Gen. Vir.*, 85: 1825–1832

Windle, E.M., 2006. Glutamine supplementation in critical illness: evidence, recommendations, and implications for clinical practice in burn care. *J. Burn Care Res.*, 27: 764–772

Xie, T., Q. Qiu, W. Zhang, T. Ning, W. Yang, C. Zheng, C. Wang, Y. Zhu and D. Yang, 2008. A biologically active rhIGF-1 fusion accumulated in transgenic rice seeds can reduce blood glucose in diabetic mice *via* oral delivery. *Peptides*, 29: 1862–1870

Ye, X., S. Al-Babili, A. Klöti, J. Zhang, P. Lucca, P. Beyer and I. Potrykus, 2000. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science*, 287: 303–305

Yoo, H.J., W.J. Park, G.M. Lee, C.S. Oh, I. Yeam, D.C. Won, C.K. Kim and J.M. Lee, 2017. Inferring the Genetic Determinants of Fruit Colors in Tomato by Carotenoid Profiling. *Molecules*, 22: 764–778

Young, T.E., J. Giesler-Lee and D.R. Gallie, 2004. Senescence-induced expression of cytokinin reverses pistil abortion during maize flower development. *Plant J.*, 38: 910–922

Yu, O., J. Shi, A.O. Hession, C.A. Maxwell, B. McGonigle and J.T. Odell, 2003. Metabolic engineering to increase isoflavone biosynthesis in soybean seed. *Phytochemistry*, 63: 753–763

Yu, O., W. Jung, J. Shi, R.A. Croes, G.M. Fader, B. McGonigle and J.T. Odell, 2000. Production of the isoflavones genistein and daidzein in non-legume dicot and monocot tissues. *Plant Physiol.*, 124: 781–794

Zafar, M.S., F. Muhammad, I. Javed, M. Akhtar, T. Khaliq, B. Aslam, A. Waheed, R. Yasmin and H. Zafar, 2013. White mulberry (*Morus alba*): A brief phytochemical and pharmacological evaluations account. *Intl. J. Agric. Biol*., 15: 612–620

Zaynab, M., S. Kanwal, I. Hussain, M. Qasim, A. Noman, U. Iqbal, G.M. Ali, K. Bahadar, A. Jamil, K. Sughra, N. Rehman, M. Buriro, S. Abbas, M. Ali, A.H. Alwi, M. Anwar, M.I. Khan and M. Tayyab, 2017. Rice Chitinase Gene Expression in Genetically Engineered Potato Confers Resistance against *Fusarium solani* and *Rhizictonia solani*. *PSM Microbiol.*, 2: 63–73

Zeh, M., A.P. Casazza, O. Kreft, U. Roessner, K. Bieberich, L. Willmitzer, R. Hoefgen and H. Hesse, 2001. Antisense inhibition of threonine synthase leads to high methionine content in transgenic potato plants. *Plant Physiol.*, 127: 792–802

Zhang, X.X., H. Yu, X.H. Wang, X.Z. Li, Y.P. Zhu, H.X. Li, S.J. Luo and Z.G. Yuan, 2013. Protective efficacy against *Chlamydophila psittaci* by oral immunization based on transgenic rice expressing MOMP in mice. *Vaccine*, 31: 698–703

Zhao, J., 2007. Nutraceuticals, nutritional therapy, phytonutrients, and phytotherapy for improvement of human health: a perspective on plant biotechnology application. *Recent Patents Biotechnol.*, 1: 75–97

Zhao, Z.Y., K. Glassman, V. Sewalt, N. Wang, M. Miller, S. Chang, T. Thompson, S. Catron, E. Wu, D. Bidney, Y. Kedebe and R. Jung, 2003. Nutritionally improved transgenic sorghum. *In: Plant Biotechnology 2002 and Beyond*, pp*:* 413–416. Springer, Dordrecht

Zhu, C., S. Naqvi, J. Breitenbach, G. Sandmann, P. Christou and T. Capell, 2008. Combinatorial genetic transformation generates a library of metabolic phenotypes for the carotenoid pathway in maize. *Proc. Natl. Acad. Sci.*, 105: 18232–18237

[Received 01 Jun 2019; Accepted 02 Jul 2019; Published (online) 22 Dec 2019]