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## Application of the lectin and non-lectin genes in transgenic crops

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### Abstract

Agricultural and horticultural crops are attacked by a number of pests, the most common of which are insect mites and nematodes, which cause damage to the plants both directly and indirectly via the fungal, bacterial, or viral infections they spread. Traditionally, agrochemicals (pesticides) were used to protect crops from pests, which had negative effects on crop yield as well as contaminating our air, affecting plant, animal, and human health. Transgenic crops that are resistant to major insect pests were one of the first achievements of plant biotechnology as a result of insects' ability to develop resistance to single insecticidal gene products. Plant with single insecticide bacillus thuringiensis and lectin genes with resistance to major pests of rice, Maize, Tobacco, and Cotton, made up the first generation of products. The objective of this review was to discuss the application, potential, and limitation of different insect-resistant genes in transgenic crops.

**Keywords:** Bacillus thuringiensis, lectin, pest, transgenic plant

### 1. Introduction

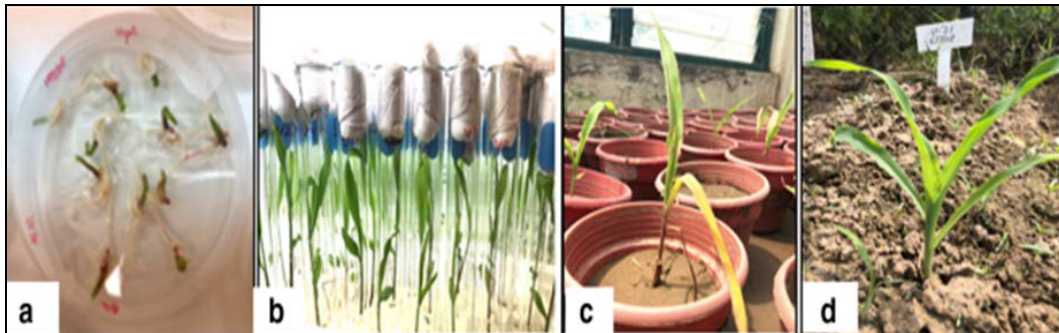
One of the most common and controversial biotechnology applications is transgenic crops [1, 2]. To decrease dependency on insect killer sprays, researchers genetically engineered cotton and corn plants to manufacture insect killer proteins determined by genes from the communal bacterium *Bacillus thuringiensis* (Bt) [3]. These Bt proteins kill some of the world's most deadly insect pests while causing minimal maltreatment to other animals, as well as humans. 4.5 out of 10 Bt crops have many benefits, including decreased pesticide use, pest control, defense of advantageous natural competitors, improved yield, and higher agronomist income [4, 5]. Bt crops have been cultivated on more than 420M hectares worldwide, up from 1.1M hectares in 1996 to 66 million hectares in 2011. Bt corn reported for 67% of corn planted in the US in 2012. Biotech Crops, Bt cotton reported for 79–95% of cotton planted in US, India, China and Australia between 2010 and 2012. The notable capability of insects to respond to pesticides and other control measures confirms the supposition that pest adaptation poses the greatest risk to the success of Bt crops [68, 9]. Pollutants resultant from bacterium, Bt Berliner are present in all insect resistant transgenic crops currently on the market [10], but transgenic crops expressing plant-derived proteins, Snowdrop lectins *Galanthus nivalis* agglutinin (GNA) are currently being studied. GNA is a lectin that binds alpha-D-mannose specifically and is toxic to a variety of insect pests from various orders, including Homoptera [9], Coleoptera [10], and Lepidoptera [11]. Potato, tobacco, wheat, rice, and sugarcane are among the crop species for which transgenic lines expressing GNA have been created. According to reports, most lectin genes have different levels of gene expression to cop various abiotic pressures, such as cold, heat, drought, and salinity [12]. Both lectin genes encoded by the rice, soybean and *Arabidopsis* genomes were recognized and characterized [13]. However, there is no comprehensive review article that has summarized combine the application, limitation and potential of the lectins and Bt genes in different transgenic plant.

### 2. Lectins application in transgenic crops

Plant Lectins have also been effectively used to protect crops from pest vermin [14, 15]. Coleoptera, Lepidoptera, and Diptera have also been found to be toxic to lectins [15]. Used Plant lectins to fight sap-feeding insects going to the Hemiptera direction, which contains around the world's record dangerous vermin.

Lectins allow nutrient interest to be blocked or midgut cells to be impaired by facilitating phagocytosis and potentially other lethal metabolites found in the hindgut<sup>[15]</sup>. Other non-Bt genes and Plant lectins have been shown to be effective against sucking insect pests in transgenic crops (Fig.1 (a-d)). Plant-derived RNA interference (RNAi) technology has occurred as a new prospect in the fight against insects,

particularly in the fight against resistance in targeted insect pests, as an alternative to conventional methods of attaining resistance, such as the use of lectins, poisonous proteins or inhibitors<sup>[16]</sup>. RNAi was first discovered in *Caenorhabditis elegans*<sup>[17]</sup> and has since proved to be an effective gene silencing mechanism in a number of organisms<sup>[18]</sup>.



**Fig 1:** Transformation steps to generate potential transgenic maize plantlets. a): germinating immature maize embryos after co-cultivation with *Agrobacterium* harboring pCAMBIA-UASAL recombinant plasmid. b): Maize transformants growing in test tubes, c-d): Hardening of transformed maize plantlets in soil pots and later in greenhouse. Source<sup>[49,50]</sup>.

### 3. Toxicity of plant lectins towards Mammals

Many plant lectins are present in a wide range of vegetables/crops (e.g. tomato, potato, pea, bean, garlic, leek, lentil, soybean, peanut, rice, corn, wheat) and fruits (e.g. banana, mulberry, breadfruit), and are consumed by humans and animals on a regular base. Since many of these plants are eaten raw, these plant lectins are considered to be non-toxic for humans and mammals in general. However, some legume lectins e.g. Concanavalin A (ConA) and Phytohaemagglutinin (PHA) are known to be toxic for mammals<sup>[19]</sup>. For example, PHA was shown to be toxic for humans especially when kidney beans were not sufficiently cooked before consumption. The acute symptoms of PHA poisoning are nausea, vomiting or diarrhea and are most likely due to the ability of PHA to bind to the epithelial cells from the digestive tract which can cause changes in cellular morphology and metabolism. It should be noted that several lectins will survive digestion by gastrointestinal enzymes. Consequently, the interaction of these plant lectins with glycoproteins in the digestive tract was reported to result in both local and systemic reactions<sup>[19]</sup>. Although toxicity was clearly shown for the broad bean (*P. vulgaris*) lectin considerable variation in lectin activity was observed for different beans<sup>20</sup>. Interestingly, the bioactivity of some plant lectins against mammalian tissues and cells could also be exploited for other applications, e.g. the use of plant lectins as potential anticancer drugs<sup>[20]</sup>. Other well-known examples of plant lectins with a severe toxicity towards mammals are ricin and abrin present in castor beans (*R. communis*) and the seeds of *Abrus precatorius* (jequirity bean), respectively<sup>[21]</sup>. However, it should be mentioned that not all ricin-B lectins are equally toxic as ricin and abrin. It has been clearly shown that ricin-B lectins from elderberry (*Sambucus* sp.) can be considered as virtually non-toxic compared to ricin<sup>[22]</sup>. Lectins related to the snowdrop lectin GNA have been studied in detail for their activity on insects. One of the major reasons for this large interest in GNA-related lectins is that several of these lectins are found in edible plants (e.g. leek, garlic), which will reduce the problems related to consumer acceptability whenever these lectins would be used in crop plants. A

report by<sup>[23]</sup> reported the binding of the snowdrop lectin to human white cells. However, these data are contradicted by other studies reporting very low if any mitogenic and immunogenic activity of GNA<sup>[24, 25]</sup>. Since the proliferative response of the GNA-related lectin from daffodil was shown to be age-related with weak mitogenicity observed for adult human lymphocytes but more than sevenfold increased effects on lymphocytes from umbilical cord blood, it is important to check different age groups when testing the response of lectins on cells<sup>[26]</sup>. Obviously, health safety assessment for each lectin is necessary before plant lectins could be introduced into crop plants for commercial purposes. In a 90-day feeding study with rats designed to assess the safety of genetically modified rice expressing the kidney bean lectin PHA-E, clear abnormalities were observed in rats after PHA-E ingestion<sup>[27]</sup>. In contrast, a similar 90-day feeding study using transgenic rice expressing GNA revealed no adverse effects on rats after continuous dietary GNA uptake<sup>[28]</sup>.

### 4. Worldwide Bt crops to pest resistance

Recent biotechnology breakthroughs have had a significant effect on agricultural crops improvement by integrating genes from different origins to establish insect pest resistance<sup>28</sup>. Pest vermin and pathogens, as previously said, are significant intimidations to crops, causing a 37% loss of productivity, with 13% of that loss due solely to pest vermin<sup>29</sup>. Since 1996, protected transgenic crops from pests, also recognized as Bt crops, have been cultivated all over the world, proving to be effective at managing pest vermin and dropping the use of toxic pesticides<sup>[30, 31]</sup> (Table 1). Insecticidal proteins produced by Bt bacteria are identified as Cry toxins because they form mineral presences. Based on primary sequence similarities, Cry toxins are grouped into fifty-four types (Cry1–Cry54) and various subtypes (e.g., Cry1Ba and Cry1Aa). They are particularly specialized in which they solitary affect a few insects, including lepidopteron, coleopterans, and dipterans, as well as nematodes<sup>[32, 33]</sup>. Although here are other families of Cry proteins that are not 3D-Cry, the three domain (3D)-Cry family is a wide group of Cry-toxins with members that are

similar in sequence and structure. Contempt the high amount of Cry toxins, only a few hundred are commercially available as sprays or in Bt crops (Cry1Ac, Cry1Ab,

Cry1Aa, Cry1F, Cry1E, Cry1D, Cry1C, Cry3B, Cry3A, Cry2Ab, Cry2Aa, and Cry34/Cry35)<sup>33</sup> (Table 2).

**Table 1:** Plant lectins have been used in a number of ways to cultivate insect-resistant crops. Pests that have been addressed as well as transition methods are explored. White backed plant hopper (WBPH), Brown plant hopper (BPH), small brown plant hopper (SBPH) and green leaf hoppers (GLH) are the four types of plant hoppers.

Crops	Gene	Application	Method	References
Cotton	Bt Vip3Aa	against major insects	Agrobacterium-mediated genetic alteration	[50]
Bamboo	Dirigent-jacalin	resistance to biotic and abiotic stresses	Agrobacterium-mediated genetic alteration	[51]
Rice	GNA	against the insect	Agrobacterium-mediated genetic alteration	[52]
Rice	ASAL	sap-feeding insects	Agrobacterium-mediated genetic alteration	[53]
Tobacco	ASAL	Homopteran insects	Agrobacterium-mediated genetic alteration	[54]
Maize	GNA	Aphids	Agrobacterium-mediated genetic alteration	[55]
Wheat	Pap	Wheat Aphids	Biolistic alteration	[56]
Tobacco	<i>lec-s</i>	Pathogens and pests	Agrobacterium-mediated genetic alteration	[57]
Tobacco	ASAL, ASAIL	cotton leaf worm	Agrobacterium-mediated genetic alteration	[58]
Wheat	GNA	Aphid Sitobion avenae	Biolistic alteration	[59]
Potato	GNA	Aphids	Agrobacterium-derived genetic alteration	[49]
Maize	ASAL	sap-feeding insects	Agrobacterium-derived genetic alteration	[60]
Onion	GNA	Aphid Colonization	Agrobacterium-derived genetic alteration	[61]
Potato	ConA	Peach-potato aphid	Agrobacterium- derived genetic alteration	[62]
Wheat	GNA	Grain aphid	Biolistic	[59]
Maize	GNA	Corn leaf aphid	Agrobacterium- derived genetic alteration	[54]
Chickpea	ASAL	Cowpea aphid	Agrobacterium- derived genetic alteration	[63]
Cotton	ACA	Cotton aphid	Agrobacterium- derived genetic alteration	[64]
Cotton	ASAL	Jassid and whitefly	Agrobacterium- derived genetic alteration	[65]
Indian mustard	ASAL	ACA (Amaranthus caudatus agglutinin) ACA-ASAL	Agrobacterium- derived genetic alteration	[66]
Indian mustard	(ACA-SAL)	Giving resistance against mustard aphid by reducing survival and fecundity	Agrobacterium-mediated genetic transformation of the apical meristem	[60]

**Table 2:** Practical resistant Bt crops

S.no	Crop	Toxin	Country	Y. marketed	Dose	Insects	References
1	Corn	Cry1Ab	S.africa	1998	Low	B.fusca	[67, 68]
2	Corn	Cry1Ab	USA	1996	Low	H.zea	[69, 70]
3	cotton	Cry2Ab	India	2006	Low	p.gossypiella	[71]
4	Corn	Cry1A105	Argentina	2010	Low	D.saccharalis	[72, 73]
5	Corn	Cry1F	USA	2003	Low	S.prugiperda	[74, 75]
6	Corn	Cry1A.105	USA	2010	Low	H.zea	[76]
7	Corn	Cry3Bb	USA	2003	Low	D. v. virgifera	[77, 78]
8	Corn	Cry1F	Brazil	2009	Low	S.prugiperda	[79, 80]
9	Cotton	Cry2Ab	USA	2003	Low	H.zea	[69, 81]
10	Corn	eCry3.Ab	USA	2014	Low	D.v. virgipera	[82, 83]
11	Cotton	Cry1Ac	USA	1996	Low	H.zea	[69, 70]
12	Corn	Cry1Ab	Brazil	2008	Low	S.prugiperda	[84]
13	Corn	Cry34/35Ab	USA	2006	Low	D.v. virgipera	[78, 85]
14	Cotton	Cry1Ac	India	2002	Low	P. gossypiella	[86, 87]
15	Corn	mCry3A	USA	2007	Low	D.v. virgipera	[78, 88]
16	Corn	Cry1Fa	USA	2003	Low	S. albicosta	[89, 90]
17	Cotton	Cry2Ab	Australia	2004	High	H. armigera	[91, 92]
19	Cotton	Cry1Ac	Brazil	2013	Low	C. includes	[93, 94]
20	Cotton	Cry1Ac	USA	1996	High	H. virescens	[70, 95]
21	Corn	Cr1Ab	Spain	1998	High	S.nonagroides	[96]
22	Cotton	Cry1Ac	China	2000	High	P. gossypiella	[97]
23	Corn	Cry1Ac	USA	1999	Low	D.grandiosella	[98, 99]
24	Corn	Cry1Ab	Spain	1998	Low	O. nubilalis	[99, 100]
25	Cotton	Cry1Ac	Australia	1996	Low	H. armigera	[101, 102]
26	Cotton	Cry1Ab	USA	2003	High	P. gossypiella	[103, 104]
27	Cotton	Cry1Ac	China	2000	High	O. nubilalis	[105, 106]
28	Cotton	Cry1Ab	Australia	2004	High	H. armigera	[91, 92]
29	Cotton	Cry1Ac	Mexico	1196	Low	H. virescens	[95]
30	Cotton	Cry1Ac	USA	1996	High	P. gossypiella	[104]
31	Cotton	Cry1Ac	Australia	1996	Low	H. punctigera	[104]
32	Corn	Cry1Fa	USA	2003	Low	O. nubilalis	[105, 106]
33	Corn	Vi3pA	Brazil	2010	High	S. frugiperda	[107]
34	Corn	Cry1Ab	USA	1196	Low	O. nubilali	[108, 109]

## 5. Limitation and risk of insect's resistance transgenic crops

Plant biotechnology has made significant progress in recent years, posing both prospects and threats. Transgenic and non-transgenic crops are grown in close vicinity. Insect movement from scattered arenas to transgenic crops may occur, and the increased pest weight that results can perimeter the benefits of transgenic crops. For several years, Bt toxins have been commonly used as "natural" pesticides, with no evidence of insect species developing resistance of their own<sup>[34]</sup>. Though, with the rapid rise in the prevalence of Bt toxins in the environment (due to transgenic crops), insect species could be under more strain to develop resistant biotypes. The indication on these problems is still unsatisfying, and careful monitoring is needed before large-scale transgenic crop deployment under subsistence farming conditions. One strategy for addressing these issues is to create a new group of transgenic with improved genes and to use gene groupings to slow down the emergence of resistance in insect inhabitants. (1) concert limits, (2) secondary pest complications, (3) insect compassion, (4) progress of evolution and resistance of new biotypes, (5) ecological effects on gene expression, (6) gene leak into the atmosphere, (7) possessions on non-target organisms, and (8) bio-safety of food from transgenic crops are all issues that bound the utility of transgenic crops for pest control<sup>34</sup>.

## 6. Plant genetic transformation Procedure, methods and their limitation

Indirect and direct transformation are the two most popular approaches for genetic transformation<sup>[35]</sup>. Indirect methods, which use bacteria, are discussed to as biological, whereas direct approaches, which depend on the dispersion of the cellular wall, are referred to as physical. Even though indirect approaches are still more common for plant alteration than direct approaches, physical approaches have lately become more popular. Indirect transition strategies use bacteria skilled of passing genes to higher plant species to insert plasmids, which are isolated circular DNA molecules present in bacteria that are distinct from the chromosome of bacteria into the target cell. *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens*, two soil innate bacteria, are the most commonly used microorganisms<sup>[36-40]</sup> and<sup>[41, 42]</sup>. A plasmid used for transformation can be somewhere between 5 and 12 kb pairs in size<sup>[43]</sup>. Plasmids contain several genes, pretend similarly to bacterial chromosomes, and are self-replicating means that they can reproduce independently inside the host. A single cell may contain up to fifty plasmids. *Agrobacterium* (Ag) can transmit an oncogene plasmid to its host and promote tumor growth<sup>[43]</sup>. This stuff (plasmid) has been used as a biotic path for genetic plant transformation, but the oncogene has been deleted (deactivated) from existing vectors, so they are no longer capable of inducing tumors. Despite its problems with regeneration of certain plants, Ag has been common in the industry<sup>[44]</sup> since the first active gene supplement in the 1980s<sup>[45]</sup>. It is broadly used for a variety of applications, but it is incomplete by the low competence of Ag transformation, mainly in monocot such as *mueslis*<sup>[46, 47]</sup>. Furthermore, Ag can familiarize vector sequences that aren't needed for transformation but may have unintended consequences in the plant<sup>[47, 48]</sup>.

## 7. Conclusion

In this study, the applications, limitations and potentials of the lectins and non lectins genes worldwide in transgenic

plants were discussed. Traditional plant breeding played an important role in crop improvement in previous decades, but the introduction of genetic engineering technology revolutionized breeding methods by breaking down hybridization barriers between species and genera. The 36th anniversary of transgenic technologies for the production of genetically engineered plants is approaching. Insect pests have had a major impact on the production of farm crops all over the world. In terms of crop production and economic benefits to farmers, the commercialization of insect-resistant crops expressing Bt and lectin genes has been excellent. It's worth noting that almost all commercially available insect-resistant crops carry Bt genes. In light of the increased production of insect resistance, it is critical to look at other causes of pest resistance in addition to implementing resistance-delaying strategies.

**8. Significance Statement:** This manuscript thoroughly covers the applications and importance of the lectins, the genes associated with these proteins and as discussed as prospective biocontrol gene targets in the efforts to make transgenic plants. Different variants of the lectins are produced/synthesized by the plants in different organs most specifically seeds, roots and leaves which are primary targets of the different insects and pathogens. Recombinant DNA technology could be employed to produce transgenic plants which will not only protect plants but also be helpful to minimize the toxic effects of agrochemicals on soil and environment.

**9. Conflicts of interest:** There are no conflicts of interest declared by the writers.

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