The Planta RBS: Vol.-2 Issue-1 (April) 2021: 279 - 292
(Research Book Series – www.pgrindias.in)

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CURRENT STATUS OF TRANSGENIC DEVELOPMENT IN PULSE CROPS#

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Abstract

Pulse crops are an integral part of sustainable agricultural production. Chickpea and pigeonpea are most important pulse crops in India because of its economic importance and nutritive value. These crops suffer significant yield losses due to various pathogens, insects , drought and salinity. In traditional breeding approaches we are not able to incorporate many useful traits in elite cultivars, consequently due to lack of resistance donor against many insect-pest and disease . The transgenic technology has made it possible to transfer any desirable gene(s) from any organism to a plant species and a high level of resistance can be incorporated in elite cultivars. Use of molecular biology techniques in combination with novel gene transfer technology has broadened the gene pool of species beyond sexual cross ability boundaries. Thus any foreign gene can be transferred in a specific fashion. The transgenic technology promises to improve crop productivity through complimenting with traditional breeding by decreasing dependence on harmful chemicals, pesticides and fertilizers.

#General Article

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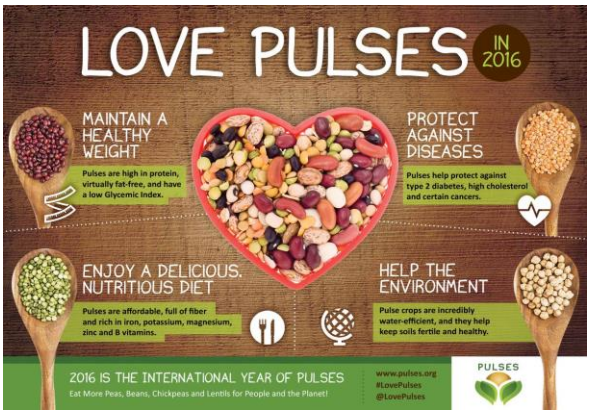
Introduction

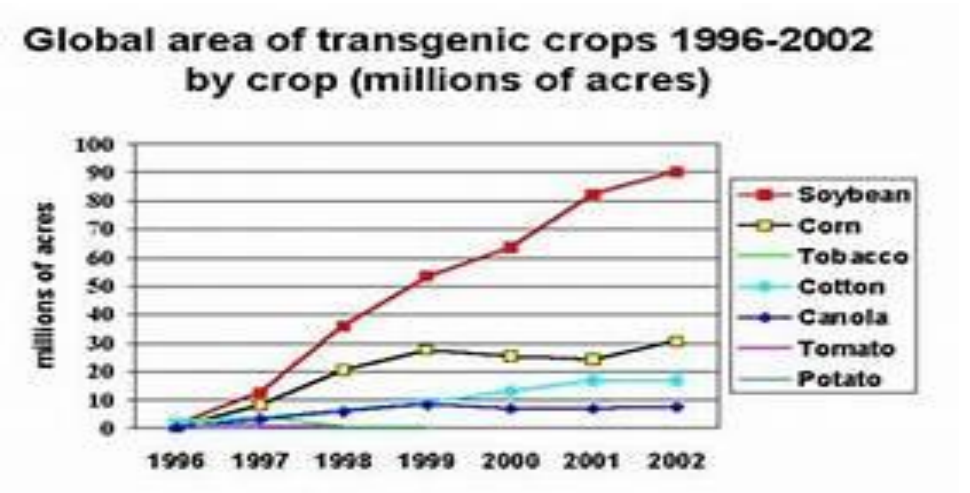


Pulses were earlier considered to be recalcitrant species and were found not amenable to biotechnological manipulations. But rapid advances in molecular biology techniques in combination and improvement of cell culture technology have allowed introducing any foreign gene into almost all plant species. It is now possible to genetically manipulate the plant species as per requirement. The stress tolerance to breaking undesirable genetic linkage or to assemble desirable genes in specific population through the traditional breeding is laborious and time consuming process. In addition, the chances of success are quiet unpredictable. The major pulse crops under cultivation and consumption include chickpea, pigeonpea, pea, lentil, mung bean, urdbean, cornmon bean and cowpea. Their yield levels are not very encouraging and often limited by a number of biotic and abiotic stresses. The present production of pulse crops is 13-14 million tons. Present Indian population of about 1015 million is expected to rise to 1350 million by 2020. Keeping in view the dietary requirement of proteins, a minimum of 27 million tons of pulses required by 2020. This can only be achieved by expanding area under pulses, raising yield genetically and minimizing the losses caused by biotic and abiotic stresses. Pulse crops are infested by many diseases and insect-pests (Table 1). With conventional methods, the available gene pool is restricted by sexual incompatible of many interspecific and intergeneric crosses (Nisbt and Web, 1990). The new technology has substantially broadened the gene pool, and has allowed the transfer of gene governing well defined traits. Development of variety is a dynamic process and their constant improvement is required due to evolvement of new races of pathogens through mutation and recombination. The new biotechnological approaches play an important role where desirable traits are to be transferred from unrelated species (N.P. Singh et al., 2004).

Table: 1. Major Biotic Stresses Identified In Pulse Crops

Pulse Crops	Diseases	Insect-pests
Chickpea	Fusarium wilt, Ascochyta blight Rust	Pod borer, bruchids
Lentil	Rust, Fusarium wilt	-----
Pea	Powdery mildew, rust	Pod borer
Pigeonpea	Fusarium wilt, Phytophthora blight	Pod borer, pod fly
Mungbean	Mungbean yellow mosaic virus, Cercospora leaf spot	Storage pests .
Urdbean	Mungbean yellow mosaic virus, Cercospora leaf spot	Storage pests

There are various methods through which foreign genes can be incorporated into the genome of target plants. Early attempts were made towards the development of an *Agrobacterium*-based vector for the introduction of an exogenous DNA into the host genome. Some of the crops, such as pigeonpea and chickpea, were initially found difficult to transform and regenerate, but there have been significant breakthrough even in these crops, it is important to note that development of efficient transformation methods is frequently not straightforward, as it can be influenced by many variables including the crop, genetic background of the material and conditions in which the experiment is being conducted.



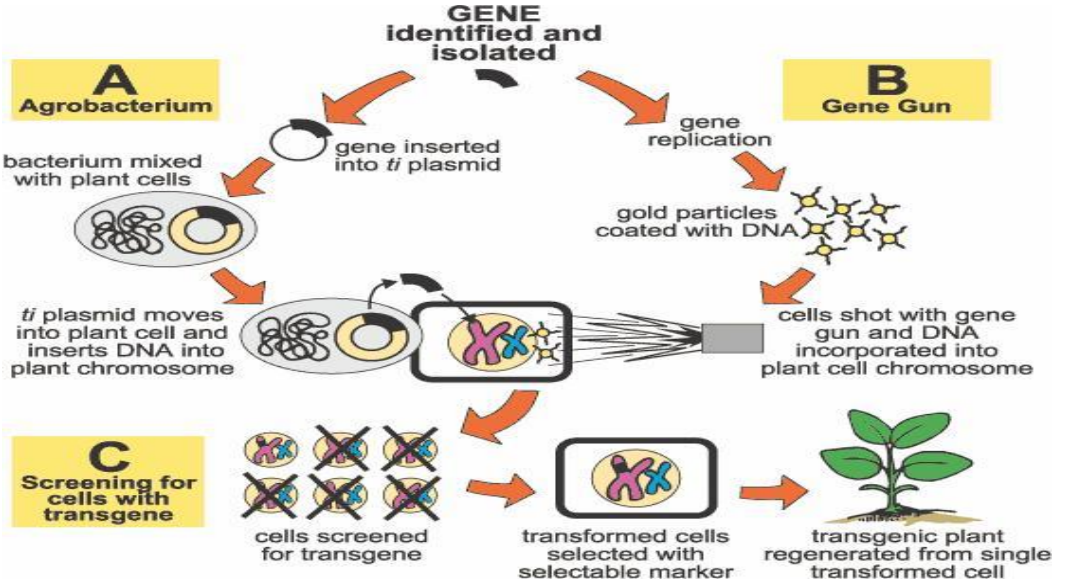
Yield Gap as per average highest yield realized in different crops

Crop	Average potential Yield (Kg/ha)	Average yield (kg/ha) of state*	Average yield (kg/ha) in FLD	National average yield (Kg/ha)
Chickpea	1800	1201	1453	1014
Pigeonpea	1800	1018	1433	776
Mungbean	1400	787	955	440
Urdbean	1300	694	883	620
Lentil	1400	941	1047	797
Field pea	2500	1315	1394	1105
Lathyrus	1200	1024	1241	742
Cowpea	1300	905	794	-
Horsegram	800	473	536	415
Mothbean	1100	-	831	280

* The average yield (kg/ha) of Chickpea and Urdbean in A.P, Pigeonpea in Gujarat, rabi/spring Mungbean and pea in UP, lentil and Lathyrus in Bihar, cowpea and Horsegram in Karnataka

Efforts are on to develop transgenic plants resistance pod borer in chickpea and pigeonpea using various forms of an endotoxin gene from *Bacillus thuringiensis* (Bt), bacterium into plants. *Bacillus thuringiensis*, a gram negative soil bacterium, has been under extensive use as a biopesticide over the past four decades in agriculture, horticulture, forestry, human and animal health. The target organ for action of Bt toxins is the insect midgut. The midgut of the lepidopteran larvae is a simple, tubular epithelium that dominates the internal architecture of the insect. The tissue is composed of two major cell types: a columner cell with a microvillate apical border and a unique goblet cell, containing a large vacuolar cavity, link to the apical surface by an elaborate and tortuous "valve". The "K^+ pump" is located in the apical membrane of the goblet cell, pumping K^+ from the cytoplasm into the cavity and then to the gut lumen via the valve. The electrogenic K+ transport is the predominant feature of the larval lepidopteran gut. Disruption of the activity of K+ pump as a result of toxin-induces pore formation in the plasma membrane of the columner cells leads to osmotic imbalance. Another important features of the midgut is that the pH of the luminal fluid is about 12, which is essential for dissolving the crystalline Bt protoxins, usually soluble only above pH 9.5. Different strains of Bt produce different insecticidal crystal proteins, coded by the *cry* genes. These crystal proteins are highly toxic to specific insects, mites, nematodes, flatworms or protozoans (Fietelson et al, 1992). Researchers are able to alter specific sequence(s) of a cry gene and fuse this altered gene to a "promoter" that allows high level of expression of Bt toxin. One can also make a "construct" with two different genes expressed under the control specific promoter. Such "synthetic Bt genes" are now more preferred by plant genetic engineers over the native (unaltered) Bt genes, as they provide greater degree of resistance to an insect pest than the native Bt genes.

All the major pulse crops can be infected by wild type *Agrobacterium* and produce tumors. So far, most of the transformations in pulse crops are limited to the transfer of marker genes, except in soybean and forage legumes. In most cases the reports do not indicate regeneration of transgenic plants and /or inheritance of the transferred gene(s) . Grain legumes are one of the least amenable groups to transformation amongst dicotyledonous crops, although they are usually susceptible to *Agrobacterium* infection. Important parameters for successful transformation of grain legumes include the characteristics of the *Agrobacterium* strain used for inoculation of target plant tissues, the vectors which the bacterial strain carries, the co-cultivation period and a selection system. Although most effort has centered upon the use of *Agrobacterium* for introducing genes into grain legumes, there are also reports of the use of biolistics. Apical meristems permit rapid multiple shoot production with minimum tissue culture compared with other types of tissues. More importantly, the genotype has less influence on plant regeneration. The transformation frequency in the case of biolistics is usually low compared to *Agrobacterium*-mediated gene transfer, however, it has been reported that particle bombardment may be the preferred option for gene introduction into large –seeded grain legumes, circumventing the host specificity of many grain legumes to infection by *Agrobacterium*.



Present Status

Development of transgenic technology has shown promises in mitigating many biotic stresses. Many transgenic varieties have already been developed. The major target insect-pests, which require priority in pulses, are listed in Table 1. Various genes are now being deployed in legume crops. The development of transgenic and expression of insecticidal resistance genes in crop plants has emerged as one of the potential methods to control insect pest. Genes from *Bacillus thuringiensis*, Bacillus sphaericus, protease inhibitors, and plant lectines have been used alone or in combination with other conventional host plant resistance to develop crop specific cultivars to provide a high level of resistance against a range of insect-pests (Hilder and Boulter, 1999; Kumar, 2003). Insect resistant Bt-transgenic crops were first grown commercially in 1996 (Krattiger, 1997). Since then the area under Bt-crops has increased steadily. In 1999, an estimated 26% of corn and 32% of cotton (Carpenter and Gianessi, 2000) grown in USA contained insecticidal protein derived from Bt. While such transgenic crops have considerable advantages both for environment and for biological safety. Efforts are on to develop transgenic plants resistance to pod borer in chickpea and pigeonpea using Bt at IIPR, Kanpur. Early result obtained are very encouraging. Similarly, efforts are also being made to develop transgenic urdbean and mungbean using coat protein gene. In chickpea few reports are available on genetic transformation. Transformated callus was obtained using wild strains of *Agrobacterium* at IARI, New Delhi. Besides transformation chickpea was reported from the same centre. However, inheritance of the transgene was not demonstrated in these studies. There is a recent report on chickpea transformation from NCL, Pune. This demonstrates transmission of a recombinant gene to the progeny for the first time besides GB Pant University, Pant Nagar and NBRI, Lucknow are also conducting research work in the same direction. At IIPR, Kanpur transformed callus and plantlets possessing nptII gene has been obtained through Agrobacterium mediated transformation.

In case of pigeonpea transformed callus and plantlets possessing foreign genes has been reported from institutes such as IARI, NBRI, NCL and Bose Institute.

Transgenic crop under development and field trials in India

Crop	Organization	Gene
Brinjal	IARI, New Delhi	*cry1Ab, cry1Ac*
	MAHYCO, Mumbai	
Cauliflower	MAHYCO, Mumbai	*cry1Ac*
	Sungrow Seeds Ltd., New Delhi	
Cabbage	Sungrow Seeds Ltd., New Delhi	*cry1Ac*
Chickpea	ICRISAT, Hyderabad	*cry1Ac, cry1Ab*
Groundnut	ICRISAT, Hyderabad	*IPCVcp, IPCV replicase,*
Maize	Monsanto, Mumbia	*CP4 EPSPS*
Mustard	IARI, New Delhi	*CodA, Osmotin,*
	NRCWS, Jabalpur	*bar, barnase, barstar*
	TERI, New Delhi	*Ssu-maize, Psy, Ssu-tpCrtI*
	UDSC, New Delhi	*bar, barnase, barstar*
Okra	MAHYCO, Mumbai	*cry1Ac*
Pigeonpea	ICRISAT, Hyderabad	*cry1Ab + SBTI*
	MAHYCO, Mumbai	*cry1Ac*
Potato	CPRI, Simla	*cry1Ab*
	NCPGR, New Delhi	*Ama-1*
Rice	Directorate of Rice Research, Hyderabad	*Bacterial blight res, Xa-21,*
	Osmania University, Hyderabad	*cry1Ab, gna gene,*
	IARI, New Delhi	*gna*
	MAHYCO, Mumbai	*Bt, chitinase, cry1Ac and Aa*
	MKU, Madurai	*cry1Ac*
	MSSRF, Chennai	*chitinase, B-1,3-glucanase*
	TNAU, Coimbatore	*chitinase*
Sorghum	MAHYCO, Mumbai	*cry1Ac*

Problems Associated with Transgenic

Genes with Insecticidal Activities

Bt Insecticidal Proteins

Bt has been the most commonly used developing insect resistant varieties. These crystal proteins are highly toxic to specific insects, nematodes, flatworms or protozoans (Fietelson et at., 1992). Bt has a wide insecticidal spectrum ranging from Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, Mallophaga and extending up to nematodes, mites and protozoa (Kumar et al.,1996; Schuler et al.,1998). Bt produces two different types of insecticidal activities which are agronomically important, the most widely known one is being called 8-endotoxin or insecticidal crystal protein (ICP). The ICP usually as protoxin of high molecular weight (135-138 kDa). Upon ingestion by insect these are proteolytically processed into small molecular weight toxin in highly alkaline midgut of the larvae. The toxin bind to specific receptor present in the membranes of midgut epithelium and cause pore formation. This causes disruption of the electrical, K + and pH gradients across the membrane leading to the death of larvae (Kumar et at., 1996). The presence of specific receptors in the classes is what determines the lack of activity of Bt 8-endotoxins towards and other organisms including beneficial insects.

Genetic engineering has facilitated stable expression of Bt genes in crop plants with considerable success (Schuler et al., 1998).

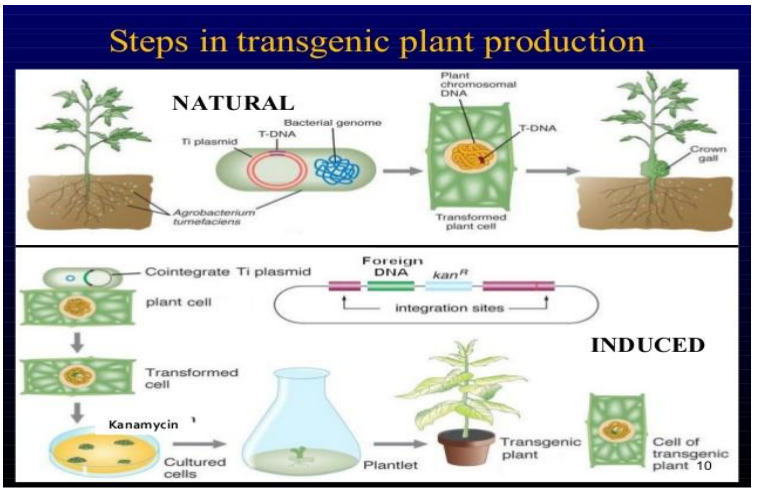
With the development of molecular methods for transfer of specific and their expression in the new host species since 1983, there is a major interest in developing transgenic plants. Transgenic technology that involves insertion of foreign DNA sequence has tremendous potential for improvement of legume plants. Both *Agrobacterium* mediated and direct gene transfer methods have been used (Table 2). All the major pulse can be infected by wild type *Agrobacterium* and produce tumors. So far, most of the transformation in pulse crops are to the transfer of marker genes, except in and forage legumes. In most cases the reports do not indicate regeneration of transgenic plants and/or inheritance of the transferred gene(s). Efforts are on to develop transgenic plants resistant to pod borer in chickpea and pigeonpea using Bt crystal protein gene. Early results obtained at various centres in the country are very encouraging. In chickpea are available on genetic transformation. Transformed callus was obtained using wild strains of *Agrobacterium* at Indian Agricultural Research Institute, New Delhi.

Table: 2. Genetic Transformation of Pulse Crops

Crop	**Type of vector**	**References**
Vigna spp	*Agrobacterium tumefaciens*	Muthukumar et al. (1996)
Pigeonpea	*Agrobacterium tumefaciens*	Lawrence and Koundal (2001)
Chickpea	*Agrobacterium tumefaciens*	Srinivasan (1991), Kar et al. (1996), Krishnamurthyet al.(2000)
Chickpea	Gene gun	Kar et at. (1997)

Besides, transformed chickpea plants possessing Cry lAc construct known for resistance to *Helicoverpa armigera* was also reported from the same centre. However, inheritance of the trans gene was not demonstrated in these studies. There is one recent report on chickpea transformation from National Chemical Laboratory (NCL), Pune. This demonstrates transmission of a recombinant gene to the progeny for the first time. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad has already made substantial progress towards development of Bt transgenic chickpea and pigeonpea. Besides, GB Pant University of Agriculture and Technology, Pantnagar and National Botanical Research Institute (NBRI), Lucknow are also conducting research work in the same direction. At Indian Institute of Pulses Research (IIPR) , Kanpur, transformed callus and plantlets possessing nptII gene have been obtained through Agrobacterium mediated transformation. In the case of pigeonpea, transformed callus and plantlets possessing foreign genes have been reported from various institutes such as IARI, IIPR, NBRI, NCL and Bose Institute.

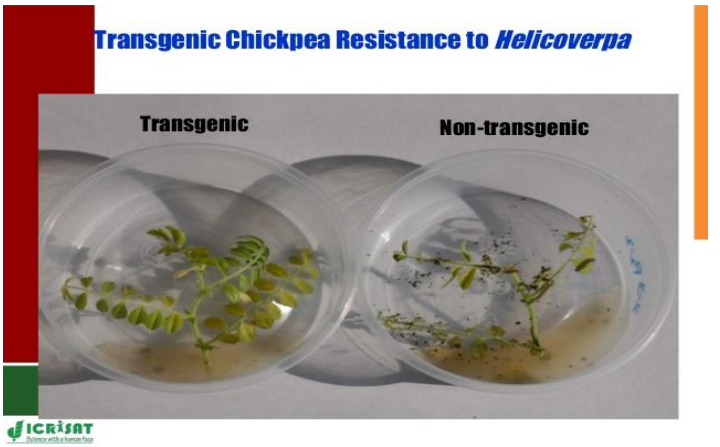
Grain legumes are one of the least amenable groups to transformation amongst dicotyledonous crops, although they are usually susceptible to *Agrobacterium* infection. Important parameters for successful transformation of grain legumes include characteristics of the *Agrobacterium* strain used for inoculation of target plant tissues, the vectors, which the bacterial strain carries, the co-cultivation period and a selection system.



Although most efforts have centered upon the use of *Agrobacterium* for introducing genes into grain legumes, there are also reports of the use of biolistic+s. Shoot apical meristems of mature seeds or whole embryos have been used extensively as target tissues for direct gene transfer by particle bombardment in *Glycine max, Phaseolus vulgaris* and with limited success in Vigna species. In the majority of the cases, explants from near the shoot apex or the apex itself have been the targets. Apical meristems permit rapid multiple shoot production with minimum tissue culture compared with other types of tissues. More importantly, the genotype has less influence on plant regeneration. The transformation frequency in case of biolistics is usually low compared to *Agrobacterium*-mediated gene transfer, however, it has been reported that particle bombardment may be the preferred option for gene introduction into large-seeded grain legumes, circumventing the host specificity of many grain legumes to infection by *Agrobacterium* .

Although many insecticidal genes have been transferred to different crop species, the most satisfactory system in terms of field resistance is the one based on Bt. Bt toxins have been expressed in at least 30 different plant species (Schuler et aI., 1998). However, the level of resistance they confer depends on whether native (wild type) or truncated, modified genes have been used (Kumar et at., 1996). The prokaryotic codon-tls-edjn Bt genes needs to be modified towards the higher plant genes. In addition, features that can destabilize the transcripts in higher plant cells need to be removed. As of today, three Bt-transgenic crops are under commercial cultivation (De Maggad et al., 1999). Bt insecticidal proteins have been expressed in soybean, alfalfa and peanut for resistance to their respective pests. A native CrylAc gene has been expressed in chickpea to confer protection against *Helicoverpa armigera* (Kar et aI., 1997). Development of pod borer larvae was affected when fed on transgenic tissues. A synthetic gene encoding CrylAc toxin was introduced in soybean by particle bombardment and the transgenic plants were observed to be resistant to com earworm (Helicoverpa zea), soybean looper (Psuedoplusia includens) and velvetbean caterpillar (Anticarsia gemmatalis) (Stewart et al., 2001). Similarly, a synthetic CrylAc gene was transferred to alfalfa for resistance to Spodoptera iittoralis (Strizhov et al., 1996). The transgenic plants produced Bt-IC to the extent of 0.01-0.2% of total soluble protein and were resistant to cotton leaf worm and beet army worm. Transformation of peanut by a synthetic CrylAc gene resulted in various levels of resistance to the lesser com stalk borer, from complete larval mortality to a 66% reduction in larval weight (Singist et al., 1997).

Protease Inhibitors



Currently, there are two major groups of plant-derived genes used to confer insect resistance on crops: inhibitor of insect digestive enzymes (proteinase and a-amylase inhibitors) and lectins. Plant protease / proteinase inhibitors are polypeptides or proteins that occur naturally in a wide range of plants and are a part of the plants natural defence system against herbivores. However, these proteins are expressed at very low levels in their native state. Hyper-expression of proteinase inhibitors in transgenic plants would lead to significant levels of insect protection. Fourteen different plant proteinase-inhibitor genes have been introduced into crop plants. Table 3 exhibits the list of legume crop species transformed with different proteinase inhibitor genes. The most active inhibitor identified to date is the cowpea trypsin inhibitor (CpTi), which has been transferred, to ten different crop species (N.P. Singh et al. 2004).

Table: 3. Insect Resistant Legume Crops

Crop	Target Pest	References
Bt insecticidal protein CrylAc		
Soybean	*Helicoverpa zea*	Stewart et al. (2001)
Peanut	*Lesser corn stalk borer*	Singist et al. (1997)
Chickpea	*Helicoverpa armigera*	Kar et al. (1996)
Alfalfa	*Spodoptera littoralis*	Strizhov et al. (1996)
Proteinase inhibitors		
Pea	*Helicoverpa armigera*	Charity et aI. (1999)
Alfalfa	*Spodoptera litoralis*	Narviez-Vasquez al. (1992)
Alfalfa	*Frcmkliniella spp.*	Thomas et al. (1994)
Bean amylase inhibitor		
Pea	*Callosobruchus maculatus*	
Pea	*Bruchus pisorum*	Shroeder et aI. (1995)
Adzuki bean	*Callosobruchus maculatus*	Ishimoto et aI. (1996)

Experiments with transgenic plants and artificial diets have sown that CpTi of Lepidopteran and Coleopteran species (Gatehouse and Hilder, inhibitors (from soybean) when expressed in transgenic tobacco and potato resulted in considerable larval mortality of Spodoptera littoralis. In addition to serine-proteinase inhibitors, one cysteine-proteinase inhibitor from rice has been introduced into several other recently; a encoding multi-

domain proteinase inhibitor precursor was in transgenic pea under the control of Rubisco small subunit promoter (Charity et al., 1999). Trials have shown that the mortality of Helicoverpa armigera larvae was as compared to controls. Protease inhibitor from insects have also been expressed in plants. In Manduca sexta (Tobacco hornworm), several protease inhibitors are present in hemolymph. One of these proteins Inhibits the activity of the enzyme elastase. Expression of this inhibitor in alfalfa has resulted in reduced thrips (Frankliniella spp.) infestation.

Production of insect resistant transgenic pea plants was first reported by Puonti-Kaerla et al. (1990) using *Agrobacterium* as a vector. The first Agrobacterium mediated transformation in lentil was reported by Warkentin and McHugan (1992).Transgenic plants have been reported for enhanced resistance against predators by expression of enzyme inhibitors. Bean α-amylase inhibitors derived from *Phaseolus vulgaris* was found to be effective against *Callosobruchus maculatus* in pea. Later this enzyme inhibitor was also reported effective against *Bruchus pisorum* and *Callosobruchus chinensis* (Shroeder et al., 1995). Similarly, tobacco proteinase inhibitor showed enhanced resistance against *Helicoverpa armigera* in transgenic peas (Charity et al., 1999).

Amylase Inhibitors

Inhibitors of a-amylases are the second type of enzyme inhibitors used to modify crop plants. a-amylase inhibitor from the common bean (*Phaseolus vulgaris*) forms a complex with and inhibits α -amylases in the midgut of coleopteran and storage pests of the genera *Callosobruchus* and blocks larval development (Ishimoto et al., 1996). Genes for three a-amylase inhibitors have been expressed in pea and Adzuki bean. The gene encoding α -amylase inhibitor under the control of a seed specific promoter in peas showed significant level of in+sect protection. Similar results were also obtained when Adzuki bean was transformed with α -amylase inhibitor gene and tested for protection against bruchid beetles (Ishimoto et al.,1996). It would be very useful if α -amylase inhibitor genes are expressed in pulses like chickpea and pigeonpea which suffer from losses due to variety of storage pests.

Lectins

Lectins are carbohydrate-binding proteins, some of which are toxic to insects. Various lectins have shown some toxicity against species of the insect order Homoptera, Coleoptera, Lepidoptera and Diptera. The mode of action of lectins against insect's remains unclear, but it has been shown that at least some binds to insect midgut epithilium cells. However, some insecticidal lectins also show significant mammalian toxicity, including lectins from *P. vulgaris*, winged bean, soybean and wheat germs. Other lectins, for example those from pea and snowdrop, have demonstrated insecticidal activity and are innocuous to mammals (Gatehouse and Hilder, 1994).

Lectins from snowdrop (*Galanthus nivalis*) have been shown to be very effective against aphids and rice brown planthopper (Powell et ai., 1995). It has been expressed in nine different crops including potato and tomato. Laboratory tests with engineered potatoes showed that snowdrop lectin did not increase the mortality or development time of potato aphid but considerably reduced fecundity (Down et al.,1996). Results of experiments with potato peach aphid were similar, but in addition, the establishment of

aphids on transgenic potatoes was reduced (Gatehouse et al.,1996). Snowdrop lectin also enhanced the resistance of potato to larvae of tomato moth (Lacanobia oleracia). The effect of snowdrop lectin is antifeedant rather than insecticidal (Gatehouse et al., 1997).

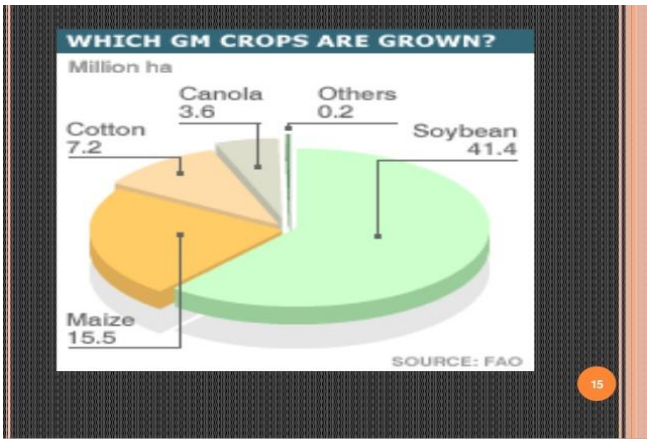
Toxins from Predators

Spiders and scorpions produce powerful neurotoxins that have in transgenic organisms (Barton and Miller, 1991). Transgenic plants of tobacco have been developed containing an insecticidal spider peptide gene, and some of plants have shown to H. annigera (Jiang et al., 1996). The role of neurotoxins from insects and spiders to be studied in greater detail before they are deployed in other organisms and plants because of their possible toxicity to mammals.

Transgenic Resistance Management

In integrated pest management, host plant resistance one of the main components. The main purpose of deployment of resistance genes plant is to manage the insect pest population and to prevent the development of resistance in insects. The insect pest management strategies are intended to prevent or diminish the selection rare individuals carrying resistance genes and hence to keep the frequency of resistance genes sufficiently at low for insect control. Strategy development generally relies on theoretical assumption and on computer models simulating insect population growth under various conditions. strategy includes the use of multiple toxins, crop rotations, high or ultra high dosages, and spatial or temporal refugia. The most promising and currently practical strategy is that of refugia.This strategy reduces the possibility of resistant insects from mating with other resistant insects, thereby preventing the creation of a resistant population. This is achieved by ensuring that there is always plenty of susceptible insects nearby for a few resistant ones to mate with. The basic principle of high dose strategy is to deploy plants with high levels of expression of toxin with the expectation that it would take a long time for insects to overcome the toxins. It assumes that most or all resistance is recessive and that most resistance carriers would be heterozygous. A viable complementary strategy that is best adopted with the above two strategies the deployment of multiple resistance or pyramiding of resistance genes. This requires more than one resistance gene with different modes of action. It could be with additional vip protease inhibitor genes or with novel methods of insect resistance, but requires the use of refugia (Gould, 1998). Targeted expression is also complementary the above described strategies and will become possible in the near future. A toxin expressed only specifically in a certain vulnerable tissue/part of the plant or is expressed both in a certain part of the plant as well as at a particular critical time in the development of the plant. This strategy would allow plenty of susceptible insects to breed normally, thus increasing predator and parasitic populations, while at the same time it is prevented from causing damage to the critical plant parts or life cycles. One of the most important tools of resistance management is to apply integrated pest management principles in transgenic crop cultivation. Use of biological control methods (predators, viruses, fungi, etc.), botanical pesticides (neem and Pyrethrum), crop rotation and sanitation, and traditional methods coupled with minimal application of chemical insecticides will prolong the life of transgenic crops.

Future Prospects



It is evident that tremendous improvements in gene transfer techniques occurred in past decade and many transgenic plants have been developed in legumes like soybean, *phaseolus*, peanut. Transgenic technology offers opportunity to avoid long gestation period to transfer the desirable genes a suitable cultivar. Current methodologies are capable only to transfer single or a few genes. The transgenic approach also provides a better solution to the problem of sexual incompatibility of interspecific and intergeneric crosses. For commercial exploitation of this technology it is necessary that it should be economically viable, environmentally safe and easy to use in diverse ecosystems. It should also be harmless to the natural enemies and nontarget organisms. It is also expected that transgenic technology should have lower risk and greater benefits than currently used alternative technologies of insect-pest management.

The tremendous improvement has place in gene transfer techniques and many transgenic plants have been developed in cereals, oilseed crops, vegetables and commercial crops. a few legumes like soybean, Phaseolus, peanut, and alfalfa could be successfully transformed. Other legumes like pea, lentil, chickpea and pigeonpea still behind as far as successful gene transfer is concerned. Apart from the most important category of Bt toxin genes, legumes have also been transformed with genes encoding protease inhibitors, a-amylase inhibitor and lectins for insect resistance. However, novel insecticidal proteins and respective need to be identified and used in conjugation with Bt to prevent development of resistant insects. Many grain legumes like pigeonpea and vigna are yet to pe successfully transformed with insecticidal protein genes, though Bt endotoxin genes are available. These legumes are relatively recalcitrant, hence, procedure needs to be developed to improve their regeneration and transformation capacity. There is also urgent need for isolation, characterization and of disease and insect-pest resistance genes from other plants and microbial sources.

Target crops for transgenic research in India

• Cereals	Rice, Wheat, Maize
• Grain legumes	Chickpea, Mungbean, Black gram, Pigeonpea
• Oilseeds	Mustard, Ground nut
• Vegetables	Brinjal, Tomato, Potato, Chilli, Cabbage, Cauliflower
• Fruits	Papaya, Banana, Muskmelon
• Medicinal plants	Brahmi
• Others	Cotton, Coffee, Tobacco

Global Status of Pulses Production 2010 (2009-10)

Crops	Area (m. ha)	Production (m.ton)	Yield (Kg/ha)
Beans (Dry)	29.88	23.23	777
Chickpea	11.99	10.94	913
Cowpeas (Dry)	10.56	5.57	527
Peas (Dry)	6.31	10.20	1616
Pigeonpea	4.75	3.68	774
Lentil	4.18	4.64	1110
Others	8.33	9.45	1134
Total	76.00	67.71	891

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