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Genetic Engineering of Crop Plants for Enhanced Resistance to Insects and Diseases in Iran

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ABSTRACT

Huge yield losses and deterioration of quality of cultivated plants occur due to continuous exposure of plants to pathogens and insect pests. The excessive use of chemical pesticides and fertilizers in modern agriculture result in a deterioration of soil fertility and through intracellular accumulation pesticide-resistant mutants of insects and plant pathogens have emerged worldwide. A number of plant species have been successfully transformed for resistance to insects, bacteria, viral, fungal pathogens and nematodes. These transgenic plants have been extensively field tested meeting the stringent biosafety guidelines and released for commercial cultivation since 1990. Genetically modified pest resistant crops have been attracting attention recently as alternatives to chemical pesticides in Iran. Iran is the first country in the region producing two transgenic plants (rice resistant to pests and cotton resistant to pests and disease) ready for release after the regulation processes. The first field trial on transgenic plants in Iran was conducted in 2004. GM rice and cotton are now under biosafety assessments. Furthermore, research on other transgenic plants such as rapeseed, wheat, palm, corn, alfalfa and potato for enhanced pest and disease resistance is underway. Currently, the National Biosafety Committee has submitted the draft of the National Biosafety Law (NBL) to the parliament for ratification. Ratification of the NBL will help to release these GM crops in the near future and modern biotechnology will certainly become one of the important components of agriculture in Iran.

Keywords: *cry* genes, chitinase, genetically modified crops, glucunase

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INTRODUCTION

By the end of the next two decades, the world will have to feed approximately 2.5 billion more people with less arable land, fewer renewable and non-renewable resources, and fewer farmers. This challenge emerges against a backdrop of rapid urbanization, climate change, globalization, and fluctuating grain prices, especially in less-developed and developing countries where the population growth will be concentrated. Conversely, the rate of production of staple food crops resulting from conventional breeding has slowed in less-developed and developing countries from 2.9% each year to 1.9%, with gains frequently being offset by environmental stresses and declining soil fertility (Bergvinson and Silverio 2004).

One way to increase the quantity and quality of crop products is to reduce damage from insects and diseases that attack crops. Such damage is commonly responsible for losses of more than 30% of the grain harvested in the less-developed and developing countries, where tropical conditions allow insects and disease agents to reproduce rapidly and to colonize unprotected crops and grains (Perlak *et al*. 1993; Raman and Altman 1994; Paoletti and Pimentel 1995).

Modern biotechnology, with an astonishing potential, is a combination of diverse related disciplines. This science finds one of its most important and promising application fields in agriculture, and even more specifically in the food industry. It is believed that modern biotechnology is one of the seven key industries, which will determine the fate of 8 billion people living on earth in 2030 (WHO 2005). The Iranian Government, understanding the importance of this science, has supported research and development in this area. In the last decade, 10 different biotechnology institutes with modern and well-equipped research facilities have been established in Iran and about 20 different universities are involved in biotechnology research. About 3000 MSc and PhD biotech-specialists are working in different research institutes and universities throughout the country. The num-

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ber of biotech companies increases every year and about 100 different private companies are presently active. Iran has achieved significant results in the area of modern biotechnology, including animal cloning (sheep) (Kazemi Ashtiani *et al*. 2008), human stem cells (Baharvand *et al*. 2007a, 2007b), plant genetic engineering (Gharayazie *et al*. 1997; Tohidfar *et al*. 2005; Salehi *et al*. 2008), and recombinant antibodies (Ismaili *et al*. 2006; Rajabi-Memari *et al*. 2006). It is the first country in the region producing two transgenic plants (rice and cotton) ready for release after regulation processes (Gharayazie *et al*. 1997; Tohidfar *et al*. 2005).

Plant diseases and insects are found as pests of crops throughout the world and especially in Iran with many having a severe economic effect. The excessive use of chemical pesticides and chemical fertilizers in modern agriculture has resulted in a deterioration of soil fertility and through their intracellular accumulation pesticide-resistant mutant insects and plant pathogens worldwide have emerged. To cope with these problems, biological control agents and genetically modified pest resistant crops have been attracting attention recently as alternatives to chemical pesticides (Mizumoto *et al*. 2006). With the recent advances of cellular and molecular biology and an understanding of the molecular mechanisms of plant-parasite interactions and disease resistance it has been possible to clone, modify and mobilize hitherto inaccessible genes from diverse sources for engineering disease and insect-pest resistant plants. Thus, applying an effective management procedure in order to preserve and protect agricultural products through exact research methods on damaging agents considering environmental factors and sustainable agriculture in Iran is the most important mission of Agricultural Research Institutes in Iran. In this report the status of application of genetic engineering of crop plants for enhanced resistance to insects and fungal disease in the world and the Islamic Republic of Iran is presented.

GLOBAL STATE OF PLANT GENETIC ENGINEERING FOR CROP PROTECTION

Genetic engineering offers new possibilities for the breeding of plant varieties with increased resistance to pests and pathogens. New resistant varieties may lessen the dependence on pesticides and help secure sufficient crop yields in the future (Stiekema 1997). Resistance of transgenic plants to insect pests or diseases has been achieved in more than 20 different crops, including maize, potato, squash, cotton, soybean, oilseed rape, tomato, tobacco, alfalfa, rice, barley and others (James 2008) (**Table 1**). These transgenic plants have been extensively field tested meeting stringent biosafety guidelines and released for commercial cultivation occupying about 40 million ha in 2007 (James 2008). Very high levels of resistance to insect pests and viral diseases have been reached, while examples of successful protection to bacterial and fungal diseases are still scarce. In the USA, permits for commercialization of more than 10 transgenic pest resistant crop varieties have been granted: Insect-resistant potato, maize and cotton expressing *Bacillus thuringiensis* (*Bt*) *crystal* toxins, virus-resistant papaya and squash expressing viral coat proteins. Several other transgenic crops are approaching commercialization. In the field of pest and disease resistance, it is likely that more insect resistant crops expressing *Bt* toxins or virus-resistant crops engineered with viral genes will enter the market in the near future. Within some years, varieties with enhanced resistance against fungal and bacterial pathogens may also become available.

Table 1 Global area of transgenic crops in 2007: by crop and traits (million ha).

Rank	Biotech crops	Area	Traits
1	Soybean	57.7	Herbicide resistance/ pest and herbicide resistance
2	Maize	19.1	Pest resistance/ pest and herbicide resistance
3	Cotton	15.0	Pest resistance/ pest and herbicide resistance
4	Canola	7.0	Herbicide resistance
5	Squash	6.2	Virus resistance
6	Tomato	3.8	Virus resistance
7	Petunia	2.6	Flower color
8	Poplar	1.8	Pest resistance
9	Sweet pepper	0.5	Virus resistance
10	Carnation	0.3	Flower/color/herbicide resistance
11	Papaya	0.1	Virus resistance
12	Alfalfa	0.1	Herbicide resistance

Source: James 2008 (ISAAA)

Insect resistance has mostly been obtained by using a gene derived from the common soil bacterium *B. thuringiensis*. This bacterium produces a protein called δ-endotoxin which is toxic to certain insects. Intensive investigations have led to a detailed knowledge of the mechanism and specificity of toxin activity. In several studies, no effect of *Bt* toxins on humans, other mammals, and most non-target insects could be shown. Transgenic plants expressing *Bt* toxin were found to be protected against repeated heavy infestations of the target insect pest which totally devastated non-transgenic control plants (Losey *et al*. 2004; Salehi Jozani *et al*. 2005, 2008).

Virus resistance is mostly achieved by introducing gene sequences derived from pathogenic viruses into the crop genome using gene silencing, antisense RNA and RNAi techniques. The introduction of genes coding for viral coat proteins has been very successful. During the last years, this strategy has led to a number of crop varieties resistant to important plant viruses, such as tomato, potato, carrot and wheat (Bucher *et al*. 2006; Ramesh *et al*. 2007; Yan *et al*. 2007). More recently, also other viral genes were found to confer resistance, e.g. replicase and RNA polymerase genes, defective viral genes or antisense coat protein genes. The mechanisms of resistance are not yet completely understood (Pandey *et al*. 2008a, 2008b; Vassilakos *et al*. 2008).

Strategies applied to achieve fungal resistance make use of plant genes acting on different levels of the plant defense system against pathogens. Several of these strategies have led to increased resistance, but so far the level of protection was mostly too low to be of agronomic importance. Chitinase and glucanase genes coding for enzymes which break down fungal cell walls have been used in several crops and have led to significant protection in some cases (Jongedijk *et al*. 1995; Owen *et al*. 2008). Some other strategies, such as suppressed expression of genes encoding allene oxide cyclase and phytodienoic acid reductase, mustard defensin gene, transgenic plants expressing harpin protein, expression of defence-related peroxidases in transgenic plants, expression of bacterial flagellin genes for plant immune responses and conferring disease resistance and overexpression of endogenous diacylglycerol kinase genes in plants, are currently used for these purposes. The growing understanding of plant defense mechanisms is expected to lead to increased levels of protection in the near future (Li *et al*. 1999; Schweizer *et al*. 2008; Swathi *et al*. 2008; Takakura *et al*. 2008; Yara *et al*. 2008; Zhang *et al*. 2008).

Also methods investigated to obtain resistance to bacteria have not led to high levels of protection yet. Reduction of disease development in tobacco and rice was achieved by transferring a cecropin gene derived from the giant silk moth (*Hyalophora cecropia*). Cecropins are produced by insects to fight pathogen attack and have a similar effect in some plants (Huang *et al*. 1997; Sharma *et al*. 2000).

Other partially successful strategies for disease resistance make use of genes which code for toxin detoxifying enzymes or plant genes involved in the response to pathogen attack. For example, *Sclerotinia sclerotiorum* causes a highly destructive disease in oilseed rape (*Brassica napus*). Oxalic acid (OA) secreted by the pathogen is a key pathogenicity factor. Oxalate oxidase (OXO) can oxidize OA into CO_2 and H_2O_2. Dong *et al*. (2008) showed that transgenic oilseed rape constitutively expressing wheat (*Triticum aesti-*

Table 2 Major plant insect pests and diseases agents in Iran.

Crops	Major pests		Major diseases	
	Common name	**Scientific name**	**Common name**	**Scientific name**
Rice	Striped stem borer	*Chilo suppressalis*	Rice blast	*Pyricularia grisea*
	Yellow stem borer	*Scirpophaga incertulas*	Sheath blight	*Rhizoctonia solani*
	Leaf folder	*Cnaphalocrocis medinalis*		
Cotton	American bollworm	*Helicoverpa armigera*	Verticillium wilt	*Verticillium dahlia*
	Spotted bollworm	*Earias insulana*	Fusarium wilt	*Fusarium oxysporum*
				F. vasinfectum
Wheat	Pink bollworm	*Pectinophora gossypiella*	Yellow rust	*Puccinia striiformis*
	Locust	*Dociostaurus maroccanus*	Leaf rust	*Puccinia recondite*
	Sunn pest	*Eurygaster integriceps*	Black rust	*Puccinia graminis*
			Fusarium head blight	*F. graminearum*
			Smuts and bunts	*Tritici tilletia*
Canola	Flower beetle	*Epicometis hirta*	Black leg	*Leprosphaeria maculans*
			Sclerotinia stem rot	*Sclerotinia sclerotiorum*
Potato	Colorado potato beetle	*Leptinotarsa decemlineata*	Common scab of potato	*Rhizoctonia solani*
				Streptomyces turgidiscabies
Maize	Iranian stem corn borer	*Ostrinia nubilalis persica*	Root rot	*Pythium graminicola*
	Lesser armyworm	*Spodoptera exigua*	Downy mildew	*Sclerospora graminicola*
	Cutworm	*Agrotis segetum*	Common smut	*Ustilago maydis*
Sugarbeet	Beet army worm	*Spodoptera exigua*	Powdery mildew	*Erysiphe betae*
	Cutworm	*Agrotis segetum*	Downy mildew	*Peronospora schachtii*
	Sugarbeet moth	*Scrobipalpa*	Bacterial root rot	*Erwinia carotovora*
Citrus			Lime witches broom	Candidatus *Phytoplasma aurantifolia*
			Citrus tristeza virus	*Closterovirus CTV*

Source: Plant Protection Organization of Iran (2007).

vum) OXO displays considerably increased OXO activity and enhanced resistance to *S. sclerotiorum* (with up to 90.2 and 88.4% disease reductions compared with the untransformed parent line and a resistant control, respectively).

STATE OF PLANT PESTS AND PATHOGENS IN AGRICULTURE OF IRAN

Diseases and insect pests limit crops yields and cause significant damage in Iran every year. Diseases and pests differ considerably in the magnitude of yield loss that they cause and in their prevalence across the different provinces of Iran. Therefore, it is important to consider regionally important diseases and pests when selecting plant varieties. With increase in disease and pest problems and resultant indiscriminate use of fungicide pesticides there is concern of environmental problems and ecological imbalance. Based on official reports of Plant Protection Organization (PPO) of Iran in 2007, about 607 different pests (50%), diseases agents (27%) and weeds (23%) caused damage to agriculture in Iran (Annual report of PPO, 2007). Iran consumes annually more than 25,000 tones of different pesticides in agriculture, of which most are used on the wheat, rice and cotton for the control of Sunn pests, stem borer and sucking pests and bollworms (Annual report of PPO, 2007). Other key pests of similar importance are yellow stem borer in rice, stem borers of sorghum and maize, fruit and shoot borer of vegetables, fruit borer of tomato and diamond back moth of cruciferous crops, cabbage and cauliflower. These pests are perennial and persistently causing losses to these economically important crops. The most important plant pathogens in Iran are blast agents, head and sheat blight agents, Verticilliums, rusts agents, Fusariums, Phytoplasma, Sclerotinia and *Citrus* viruses. Farmers are unable to control these pests to desired level in spite of spending millions of dollars on pesticides. One of the most important national priorities in the country is reduction of plant pesticide use nation wide, stopping illegal importation of pesticides, elimination of obsolete pesticides, and finally finding new environment friendly strategies for control of plant pests. Genetic resistance to diseases and insect pests is usually the most effective, most economical, and most environmentally sound method of control. As one possible alternate strategy to chemical pest control, genetically engineered crops and microbial biocontrol methods can be used due to their effectiveness. **Table 2** briefly shows the key pests and diseases cause significant economical damage in Iran.

STATE OF PLANT GENETIC ENGINEERING FOR CROP PROTECTION IN IRAN

Bt rice resistant to striped stem borer

Rice is used as a staple food in Iran, and about 640,000 hectares are under rice cultivation in Iran where two million tons of husked rice are produced annually. The official statistics emphasize that Iran needs to import 450,000 tons of rice per annum at present. Rice is susceptible to several insect pests, including striped stem borer (SSB; *Chilo suppressalis*) the major insect pest of rice in Iran. Estimated loss due to the damage caused by this insect ranges between 5 and 20%. There is no known source of resistance against stem borers in the world collection of rice germplasm maintained at the International Rice Research Institute (IRRI). In collaboration with IRRI a synthetic *cry1A(b)* gene was introduced to Iranian variety Tarom Molaii using the biolistic gun approach. Embryogenic calli derived from mature seeds were bombarded with gold particles coated with plasmid pCIB4421, carrying a synthetic truncated toxin gene, and plasmid pHygII, carrying the hygromycin phosphotransferase (*hpt*) selectable marker gene. Line No. 827 produced truncated (67 kDa) CryIA(b) protein equivalent to about 0.1% of total soluble protein. The *cryIA(b)* gene was controlled by the promoter of the maize C_4 PEP carboxylase gene and was expressed in leaf blades but was not expressed to a detectable level in dehulled mature grain. Line 827 contained about 3 copies of the *cryIA(b)* gene which segregated as a single dominant Mendelian locus in the second (T_1) and third (T_2) generations and co-segregated with enhanced resistance to first-instar larvae of striped stem borer (*Chilo suppressalis*) and yellow stem borer (*Scirpophaga incertulas*). Molecular analysis showed the stable integration of the gene into a single locus expression at a high level (about 0.1% of plant total soluble protein) (Ghareyazie *et al*. 1997). The stability of insect resistance in this transgenic plant has made it as an interesting genetic material to be used in different breeding programs (Ghareyazie *et al*. 1997). Different greenhouse and field trial experiments on this transgenic line showed the functionality and durability of the introduced gene.

Fig. 1 Bioassay using bollworm (*Heliothis armigera*). (**Right**) After 7 days on leaves of transgenic line, first larvae were all dead. No visible damage, no live larvae recovered. (**Left**) After 7 days on leaves of non-transgenic control plants, larvae were alive and progressed to second instar. Extensive visible damage was observed and no dead larvae were recovered (unpublished data).

Bt cotton resistant to bollworm

Cotton is an important fiber crop in Iran and is cultivated on 150,000-200,000 ha. Cotton is susceptible to several insect pests including bollworm (*Helicoverpa armigera*) the major insect pest of cotton in Iran. The estimated loss due to the insect pests is more than 30% in Iran. Traditionally, this pest is controlled by 10-12 times spraying of environmentally harmful chemical insecticides. There is no known source of resistance against bollworm in the collection of cotton germplasm. In order to produce transgenic cotton resistance to insects, hypocotyl explants were transformed with *Agrobacterium tumefaciens* strain LBA4404, harboring the recombinant binary vector pBI121 containing the *cry1Ab* gene under the control of CaMV35S promoter. The neomycin phosphotransferase (*npt*II) gene was used as the selectable marker (Tohidfar *et al*. 2005). Inoculated tissue sections were placed onto co-cultivation medium. Transformed calli were selected on MS medium containing 50 mgl^{-1} kanamycin and 200 mgl^{-1} cefotaxime. Plantlets were subsequently regenerated from putative transgenic calli. Polymerase chain reaction and Southern blot analysis were used to confirm the integration of one copy of the *cry1Ab* and *npt*II transgenes into the plant genome. Western immunoblot analysis of proteins extracted from leaves of transgenic plants revealed the presence of an immunoreactive band with MW of approximately 67 kDa in transgenic cotton lines using anti-Cry1Ab polyclonal anti-serum. Homozygous T2 plants (Line #61) for the *cryIA(b)* gene showed significantly higher levels of insect resistance against *Helicoverpa armigera* larvae compared with the control plants (**Fig. 1**) (Tohidfar *et al*. 2005, 2008). Transgenic plants will be evaluated in field trials and crossed with Iranian cotton breeding lines after approval of the National Biosafety Law by Parliament.

GM cotton resistant to *Verticillium*

Verticilliosis is a vascular fungal disease caused by *Verticillium dahlia* and is considered as a major wilt disease in cotton-growing areas in Iran. There is no known resistance source in the cultivated and wild relatives of cotton plant. The Vascular wilt diseases are particularly difficult to control because they are soil-borne and therefore difficult to treat with chemical fungicides. However, because of the wide sources of resistance in cotton, it is very difficult to transfer resistance to cultivated cotton and select cultivars with a high level of resistance to *Verticillium* wilt via conventional plant breeding. Also, it takes much time to develop resistant plants by conventional plant breeding. Therefore selection of new varieties with a high level of resistance to disease using biotechnological approaches is becoming the main target for cotton breeders. To enhance cotton resistance against this fungal disease, a bean chitinase gene was transferred to a cotton variety Cooker by *Agrobacterium* method. Homozygous T2 plants of the high chitinase-expression cotton lines containing one copy of the gene were evaluated for their tolerance to *Verticillium* in the greenhouse. Seedlings were infected with a spore suspension with a density of 10^6 spores/ ml in the greenhouse. Transgenic plants demonstrated higher levels of tolerance to the fungus compared to the non-transgenic plants, as measured by foliar disease symptoms, vascular discoloration and plant height (**Fig. 2**). Transgenic plants were taller than the control plants. Transgenic plants are currently grown in greenhouse and will be crossed with Iranian cotton breeding lines (Tohidfar *et al*. 2005). Transgenic plants will be evaluated in field trials and crossed with Iranian cotton breeding lines after approval of the National Biosafety Law by Parliament.

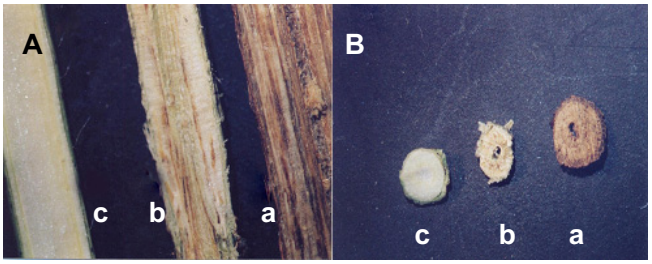

Fig. 2 Horizontal (A) and vertical (B) cutting of stem. Vascular browning in cotton plants inoculated with 10^6 conidia ml^{-1} suspension. a: untransformed plant inoculated with distilled water, b: transformed plant inoculated with 10^6 conidia ml^{-1} suspension, c: untransformed plant inoculated with 10^6 conidia ml^{-1} suspension (unpublished data).

Potato resistant to Colorado potato beetle

B. thuringiensis wild type *cry* genes have many potential splicing, transcription termination sites and mRNA degradation signals as well as codons that are rare in plant genes causing suboptimal expression of such genes in plants (Adang *et al*. 1993; Bohorova *et al*. 2001). Plants containing the wild type gene expressed Cry3a protein at less than 0.001% of total leaf protein, although *cry3a* mRNA was detectable by northern analysis (Perlak *et al*. 1988, 1993). In this study, which was performed in collaboration with laboratory of Functional Genomics of Vavilov Institute of General Genetics, Russian Academy of Sciences, using the lichenase reporter system also reported that the modified *cry3a* gene (*cry3*aM) was expressed at high levels in transgenic potato plants (up to 100-fold compared to the native gene which was studied previously, and this expression confers a high level of protection against damage caused by Colorado potato beetle (CPB) (Salehi Jouzani *et al.* 2008).

The fully-modified *B. thuringiensis cry3a* (*cry3aM*) gene was designed and synthesized for effective expression in plants. Hybrid genes *cry*3a-*lic*BM2 and *cry*3aM-*lic*BM2 were constructed, in which the sequences of the native and modified genes were fused with the reporter gene for thermostable lichenase in the reading frame. It was shown that the expression levels of hybrid genes *cry*3a-*lic*BM2 and *cry*3aM-*lic*BM2 in *E. coli* were comparable, being 5% of those for reporter gene *lic*BM2. In cells of a lower eukaryote *Saccharomyces cerevisiae*, the expression of hybrid gene *cry*3aM-*lic*BM2, which contained the modified gene, considerably exceeded the level of expression of *cry*3a-*lic*BM2 containing the native gene (Salehi Jouzani *et al.* 2005). A plant expression vector pC29RBCS-*leader-cry3aM–licBM2* was constructed for potato transformation. In this vector, the *cry3aM* sequence was fused in reading frame with a new reporter gene (*licBM2*) and a leader sequence for the *rbcs* gene. The reporter gene encoded thermostable lichenase and the leader sequence encoded a sig-

Fig. 3 Bioassay of primary transgenic potato lines using Colorado Potato Beetle (*Leptinotarsa decemlineata*). (Right) After 4 days on leaves of transgenic line with five newly hatched CPB neonate larvae, no visible damage to leaf. (Left) After 4 days on leaves of no- transgenic line with five newly hatched CPB neonate larvae. Extensive visible damage was observed and no dead larvae were recovered (unpublished data).

Table 3 Current status of plant transformation for insects and diseases resistance in Iran.

Crop	Gene/Trait	Current status		
		Laboratory	Greenhouse	Field test
Rice	*crylAb*, *insect resistance*	•	•	•
Cotton	*crylAb*	•	•	–
Cotton	*chitinase*, *disease resistance*	•	•	–
Cotton	*chitinase*, *glucunase*	•	•	–
Alfalfa	*cry3a/ insect resistance*	•	–	–
Maize	*crylAb*	•	–	–
Wheat	*glucanase*, *chitinase*	•	–	–
Sugar beet	*crylAb*	•	•	–
Canola	*agl1*, *agl5*, *agl8*	•	–	–
Canola	*Herbicide resistance gene*	•	•	–
Cumin	*gox*	•	–	–
Potato	*cry3a*	•	–	–
Date palm	*cry1Ab*	•	–	–

Large dots (•) show that experiments were performed on that level.
Dashes (–) show that the experiments were not performed on that level.

nal peptide for transporting protein product to chloroplasts. The vector contained the light-inducible promoter for the *rbcs* gene isolated from *Arabidopsis thaliana*. Microtuber explants were produced according to Ishida et al. (1989) with some minor modifications. Transgenic plants were obtained by *Agrobacterium*-mediated transformation using microtuber explants. Transgenic plants were rooted on kanamycin-containing media in two stages, first at 100 mg/l kanamycin and then at 200 mg/l kanamycin. Transgenic plantlets were confirmed as transgenic by PCR with specific primers, evaluation of lichenase activity, and bioassay of Colorado Potato Beetle (*Leptinotarsa decemlineata*) neonate larvae. Mortality rates ranged between 37 and 81%, and many surviving insects suffered from 50 to 70% reduction in body weight and failed to pupate (**Fig. 3**). Promoter activity assays under light induction (kinetic analysis) using lichenase activity and bioassay both showed high and stable expression of hybrid genes in transgenic plantlets. Furthermore, the presence of lichenase as a reporter protein in the composition of hybrid protein was shown to facilitate selection and analysis of the expression level of hybrid genes in transgenic plants (Salehi Jouzani *et al.* 2008).

Alfalfa resistant to weevil

Alfalfa (*Medicago sativa*) is an important forage crop in Iran and is cultivated on 600,000 ha. Alfalfa is susceptible to several insect pests including alfalfa weevil, *Hypera postica*, the major insect pest of alfalfa in Iran. The estimated loss due to the insect pests is more than 80% in the first harvest. There is no known source of resistance against *Hypera postica* in the collection of alfalfa germplasm. In order to produce transgenic alfalfa resistance to insects, a synthetic *cry3a* gene was transformed to Iranian variety alfalfa using *A. tumefaciens*. Transgenic plants are currently being grown in a greenhouse and will be assayed with pest insects (Zare *et al*. 2008).

CURRENT RESEARCH

Transformation of different rice varieties, cotton, sugar beet, maize, potato, barley, canola and date palm for enhancement of resistance to pests and diseases is currently conducted in different research institutes and universities of Iran using both *Agrobacterium*-mediated and biolistic approaches. The status of plant genetic engineering projects for enhanced resistance to pests and diseases is shown in **Table 3**.

CONCLUDING REMARKS

The establishment of the National Council for Scientific Research under the presidential office and ratification of National Biosafety Law have raised hopes among scientists in Iran. Strong support of biotechnology by the government, inclusion of plant breeder's right law, preparation of the draft for biosafety and increased desire of private sector for investment and involvement in biotechnology are hope-raising signals. Several transgenic crop plants with enhanced resistance to pests and diseases have been produced ready for release after approval by the National Biosafety Committee.

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