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Genetic Engineering for Disease and Pest Resistance in Plants

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Abstract

Huge yield losses and deterioration of quality of cultivated plants have been realized due to continuous exposure of plants to pathogens and insect-pests. The plants and their obligate pathogens and pests have evolved for co-existence. This natural balance has been disturbed by deployment of genes for race-specific vertical resistance and excessive use of pesticides leading to a vicious cycle of emergence of new virulences and search for new genes and potent pesticides. The genetic base for resistance to diseases and insect pests is extremely narrow or does not exist for same plant-pathogen or plant-insect combinations. With the recent advances of cellular and molecular biology and understanding of molecular mechanism of plant-parasite interactions and disease resistance it has been possible to clone, modify and mobilize hitherto inaccesible genes from diverse sources for engineering disease and insect-pest resistant plants. A number of plant species have been successfully transformed for resistance to bacteria, viral, fungal pathogens, nematodes and insects. The transgenic plants have been extensively field tested meeting the stringent biosafety guidelines and released for commercial cultivation since 1990 occupying more than 12 million hectares. Perspectives and strategies for overcoming crucial constraints and concern for engineering high levels of resistance and its exploitation are discussed.

Introduction

Most of the cultivated plants including field and vegetable crops, ornamentals and trees are continuously exposed to abiotic and biotic stresses at their different growth stages from seedlings to seeds (Dhaliwal *et al.*, 1998). Such constrains caused huge losses in economic yield and deterioration of nutritional and processing quality during post-harvest handling and storage. The biotic stress comprises of fungal, bacterial and viral pathogens, nematodes, insects and weeds. Almost all the obligate pathogens, nematodes and insects and their host plants have evolved in such a way that the co-existence of the host and the parasite is ensured. However, during domestication and subsequent improvement of plants for higher productivity per unit area while meeting the stringent quality standards, the natural balance between plants and their biotic partners is heavily tilted in the favor of former through deployment of sources of genetic resistance and application of pesticides. This has led to vicious cycle of development of new virulences and biotypes and vigorous search for new sources of resistance or potent chemicals which is a matter of universal concern for sustainability of food production, environment and biodiversity. Ever since the understanding of genetics of resistance and host-pathogen interaction, diverse sources of resistance to diseases and insect-pests have been identified from cultivated germplasm and related wild species and deployed for commercial exploitation. Unfortunately, in many host-pathogen or host-insect systems, sources of genetic resistance either do not exist or are very limited. A number of recent advances in cellular and molecular biology have opened up vast opportunities for alleviating the biotic stresses of plants by identification, isolation and *in vitro* modification of novel sources of resistances at molecular levels and their mobilization across the barriers of sexual hybridization by genetic engineering.

More than 25,000 field trials including transgenic plants for diseases and insect-pest resistance have been conducted globally from 1986 to 1997 in 45 countries involving more than 60 crops and some of which are being grown commercially occupying more than 12.8 million hectares by 1997 (James, 1997). The progress of basic and applied research

for combating biotic stresses of plants through genetic engineering has been critically reviewed by several workers (Shah, 1997, Dempsey *et al.*, 1998, Jung *et al.*, 1998, Schuler *et al.*, 1998, Jouanin *et al.*, 1998). Some of the latest developments in genetic engineering of plants for resistance to diseases and insect-pest and perspectives and strategies for further improvement have been reviewed in this article.

Molecular mechanism of host-pathogen recognition and disease resistance

The cloning of a number of *R* genes for resistance in the host plants against disease inter- and intracellular, obligate and facultative plant pathogens including bacteria, viruses and fungi and genes for avirulence (*avr*) in some pathogens has led to considerable understanding of the molecular mechanisms of plant-microbe interaction and disease resistance and formulation of strategies for engineering disease resistance (Song *et al.*, 1995, Staskawicz *et al.*, 1995, Baker *et al.*, 1997). The interaction between the elicitors coded directly or indirectly by avirulence genes with the receptors coded by *R* genes activates a cascade of host defense genes that leads to hypersensitive response (HR) and inhibition of pathogens by localized death in the host at the infection site restricting further spread. HR of plants to different pathogens includes oxidative burst, ion fluxes, cross linking and strengthening of cell wall, production of antimicrobial compounds and induction of several categories of pathogenesis related (PR) defense response genes such as chitinases and glucanases (Lamb and Dixon, 1997, Jabs *et al.*, 1997). Nitric oxide has also been reported recently to synergise reactive oxygen intermediate (ROI) in pathogen induced HR and to independently induce genes for synthesis of protective natural products (Delledonne *et al.*, 1998). HR and other necrotic reactions induce systemic acquired resistance (SAR) which operates non-specifically throughout the plant providing resistance against a wide range of pathogens. Although accumulation of salicylic acid is associated with the HR and SAR but whether salicylic acid is a systemic signal has not been unequivocally resolved. Chemical activators of SAR such as benzothiadiazole (Gorlach *et al.*, 1996) and 2,6-dicholoro isonicotinic acid (INA) have been identified. A number of lesion mimic mutants with high level of resistance to bacterial and fungal pathogens have been identified in maize and *Arabidopsis*. Similarly, *Arabidopsis* mutants in the SAR signal transduction pathway displaying constitutive expression of PR genes or deficient in SAR have been isolated. Alternative pathways for triggering SAR independent of SA and PR gene expression by non-pathogenic biocontrol bacteria has been identified in *Arabidopsis* (Pieterse *et al.*, 1996). A number of transgenic plants with resistance to diseases have been developed using the cloned R and PR genes and HR and SAR signal transduction intermediates and mutants some of which have been discussed below.

Bacterial and Fungal Diseases

Xa21, a *R* gene from a wild relative of rice *Oryza longistaminata* conferred resistance to 29 different isolates of *Xanthomons oryzae* pv *oryzae* in transgenic rice (Wang *et al.*, 1996). Similarly *R* genes such as *Bs2* from pepper and *mlo* from barley capable of providing resistance to multiple isolates of pathogens can be useful for providing broad spectrum resistance in homologous and heterologous systems (Kearney *et al.*, 1990, Wolter *et al.*, 1993). The *N* gene of tobacco provided resistance to tobacco mosaic virus in transgenic tomato (Whitham *et al.*, 1996). The tomato *Cf-9* gene providing resistance to *Cladosporium fulvum* isolates expressing the complimentary avirulence gene *Avr9* also conferred responsiveness to *Avr9* product in non-host potato and tobacco (Hammond-Kosack *et al.*, 1998). The progress of the two-component system for engineering broad-spectrum resistance based on eliciting HR in plants containing an *R* gene with pathogen-inducible expression of the cognate *Avr* gene has been reviewed elsewhere (Shah *et al.*, 1997, Dempsey *et al.*, 1998).

The direct role of ROIs in plant defense mechanism has been demonstrated by resistance to bacterial and fungal pathogens in transgenic tobacco with constitutive expression of $H_2O_2^-$ generating glucose oxidase gene from *Aspergillus niger* (Wu *et al.*, 1995). The role of low molecular weight antimicrobial compounds called phytoalexins whose synthesis and accumulation are frequently associated with HR could not be demonstrated unequivocally in transgenic plants. A number of transgenic plants expressing one or the other genes for pathogenesis related (PR) proteins have been found to exhibit partial resistance to fungal pathogens which appears to be inefficient for commercial exploitation. Combinatorial expression of hydrolytic PR enzymes chitinases and 1,3-β-glucanases in transgenic plants gave enhanced level of disease resistance (Jach *et al.*, 1995). However, transgenic tobacco and potato plants with endochitinase gene from the

mycoparasitic fungus *Trichoderma harzianum* highly tolerant or completely resistant to foliar and soil borne pathogens could be obtained (Lorito *et al.*, 1998).

Transgenic plants expressing genes including ribosome-inactivating proteins (RIPs) with 28S rRNA N-glycosidase activity also synergestically enhanced antifungal activity of chitinase and 1,3-β-glucanase (Jach *et al.*, 1995). Transgenic plants containing bacterial genes capable of inactivating or insensitive to toxins produced by *Pseudomonas syringae* pathogens have been reported to be totally resistant to the bacteria (Mourgues *et al.*, 1998).

Resistance provided by one or more PR genes is much narrower than that rendered by full-fledged SAR which is the simultaneous expression of a battery of PR genes. Identification and manipulation of genes involved in the SAR signal transduction pathway would, therefore, help in engineering non-specific broad spectrum resistance in plants. In *A. thaliana*, a gene *NPR1*, non-expression of PR genes (also called NIM1 or SAI1) has been identified as the key regulator in transducing the SA signal leading to SAR. Mutations in the *NPR1* remain susceptible to bacterial and fungal pathogens even when the plants are protected with the SAR inducers. The *NPR1* encodes a protein containing an ankyrin-repeat domain found in regulatory protein of animal immune response. The induced NPR1 protein activates a battery of downstream PR genes conferring resistance to pathogen *Pseudomonas syringae* and *Perenospora parasitica* in a dosage dependent fashion. Over expression of NPR1 in transgenic plants led to enhanced resistance with no detrimental effect on plants (Cao *et al.*, 1998) suggesting the possibility of engineering broad spectrum non-specific disease resistance.

Another novel signaling pathway controlling induced systemic resistance (ISR) by a non-pathogenic rhizobacteria *Pseudomonas fluorescens* dependent on jasmonate, ethylene and NPR1 but independent of SA has been reported in *Arabidopsis*. The downstream processes of *NPR1* in ISR pathway have been reported to be different from those in the SAR pathway indicating that the *NPR1* differentially regulates defense responses depending upon the elicited signals during induction of resistance (Pieterse *et al.*, 1998). The understanding of molecular mechanisms of the divergent downstream processes may further lead to engineer novel disease resistance mechanisms.

Viral Diseases

Several successful attempts for engineering resistance to viral diseases in transgenic plants using viral-coat-protein (CP) coding sequences; expression of antisense viral transcripts and nucleic acid encoding viral satellite RNAs have been summarized (Gadani *et al.*, 1990). Transgenic tobacco plants with full length cDNA copy of the genomic RNA of a mildly virulent tomato strain of tobacco mosaic virus (TMV-L11A) were more resistant to the highly virulent TMV-L strain than the CP-mediated transgenic plants. The engineered cross-protection was not overcome by inoculation with TMV-L RNA. Subsequently a number of transgenic plants expressing antisense viral genes and non-structural viral sequences with resistance to virus have been reported. Transgenic plants of commercial cultivar of tobacco expressing cucumber mosaic virus satellite RNA and coat protein remained symptomless upto 90 days and had resistance about twice than that conferred by the Sat-RNA and CP gene alone in the transformed plants (Yie *et al.*, 1992).

Ribozymes, the small RNA molecules capable of highly specific catalytic cleavage of RNA have enormous potential to develop virus resistant plants by inhibiting viral replication. de Feyter *et al.* (1996) found that the homozygous transgenic tobacco plants expressing ribozymes with three catalytic hammerhead domains were as resistant to TMV as the one with corresponding antisense gene. Yang *et al.* (1997) have also reported high level of resistance to potato spindle tuber viroid (PSTVd) in transgenic potato expressing the active hammerhead ribozyme R (-) targeting the minus strand RNA of PSTVd. Virus resistant transgenic plants expressing the mutant forms of viral movement proteins (MP) have been developed. Movement proteins mediated resistance conferring delayed symptoms and/or decreased systemic viral accumulations would provide much broader spectrum of virus resistance than the CP-mediated or replicase mediated resistance (Dempsey *et al.*, 1998, Beachy, 1997).

Nematode resistance

Cyst nematodes of the genera *Heterodera* and *Globodera* and root-knot nematodes of the genus *Meloidogyne* cause heavy economic losses in many crop plants worldwide. Root-knot nematodes feed from the multinucleate giant cells developed by the expansion of cambial cells within the root vascular cylinder whereas the cyst nematodes feed from a

syncytium composed of numerous cells after fusion of protoplasts (Jung *et al.*, 1998). Females finish their life cycle within the root after depositing eggs in the egg sac or cysts. Typical symptoms of nematode infection are changes in root morphology, stunted growth and yellowing. A number of genes for resistance to nematodes have been identified in different crop plants, tagged with molecular markers (Cai *et al.*, 1997, Milligan *et al.*, 1998) and isolated using map based cloning and complementation. These genes like the R gene for disease resistance exhibit the gene-for-gene relationship and elicit HR upon infection by nematode. Cystatins are known to inhibit cysteine proteinases involved in protein metabolism and digestion of dietary protein present in nematodes. Transgenic *Arabidopsis* expressing modified rice cystatin (OC-I-delta D86) were resistant to both cyst and root-knot nematodes (Urwin *et al.*, 1997). Transgenic rice plants expressing detectable levels of Oryzacystatin-I-delta D86 an engineered cystatin proteinase upto 0.2% of total soluble protein in plant roots resulted in 55% reduction in egg production of *Meloidogyne incognita* (Vain *et al.*, 1998). A few other strategies such as use of Bt-endotoxin and antibodies directed against nematode specific proteins for engineering nematode resistance in plants have also been discussed (Jung *et al.*, 1998).

Insect resistance

A number of genes of microbial, plants, and animal origin have been used for engineering plants for insect resistance (Schuler *et al.*, 1998, Jouanin *et al.*, 1998), a few of which such as Bollgard™ cotton and Newleaf™ potato have already been released for cultivation. More than 90 genes encoding protoxins from different *Bacillus thuringiensis* strains with the specificity against one or more of *Lepidoptera*, *Coleoptera* and *Diptera* insects have been cloned and sequenced. After the first successful genetic transformation of plants with Cry genes in 1987 a number of native and synthetic cry genes and their constructs have been used for optimal expression in transgenic plants (Schuler *et al.*, 1998). To have high level of expression of native cry genes in transgenic plants for effective insect control, McBride *et al.* (1995) integrated a native cry gene into the chloroplast genome of tobacco by homologous recombination with toxin production reaching upto 3-5% of the leaf soluble protein. Estruch *et al.* (1996) isolated a novel insecticide protein Vip3A from *B. thuringiensis* produced during vegetative growth of bacteria unlike the cry proteins and possessing broad insecticidal activities against lepidopteral larvae. The gene for Vip3A can be used for engineering plants for insect resistance.

Among the plant derived genes proteinase and α-amylase inhibitors and lectins have been used for engineering plants for insect resistance. Serine-and cysteine-proteinase inhibitors have been reported to inhibit the growth and development of lepidoptern and coleopteran insect species, respectively. More than a dozen different plant proteinase-inhibitor genes have been mobilized into crop plants (Schuler *et al.*, 1998). The most potent inhibitor identified so far is the cow pea trypsin inhibitor (CpTI) which has been transferred to several plant species affecting a wide range of lepidopteran and coleopteran insects. However, due to suboptimal expression and effectiveness, transgenic plants with proteinase inhibitors including CpTI have not been commercialized. Among the amylase inhibitors, main emphasis has been on the transfer of common bean (*Phaseolus vulgaris*) α-amylase inhibitor (α AI-Pv). Transgenic plants of peas and Azuki beans expressing the α AI-Pv showed resistance to one or more species of bruchid beetles (Estruch *et al.*, 1996). Some of the lectins, the carbohydrates binding proteins, abundant in the seed and storage tissues of plants show selective toxicity to insects of different orders. Transgenic potatoes expressing the snowdrop (GNA) lectin reduced fecundity of potato aphid in glasshouse trials (Down *et al.*, 1996). The insecticidal potential of a number of other genes from diverse sources has been developed (Schuler *et al.*, 1998, Jouanin *et al.*, 1998). The genes encoding the proteins could represent alternative to Bt for transgenic development. The use of insect chitinase genes alone or in combination with Bt gene for engineering insect resistance has also been advocated (Ding *et al.*, 1998). *Meu-1* providing resistance against potato aphid Macrosiphum euphoribae previously known to be tightly limited to nematode resistance gene, *Mi*, has been found to be one and the same gene as *Mi* which has been isolated earlier by positional cloning. The susceptible tomato plants transformed with Mi were resistant to certain aphid isolates thus demonstrating for the first time a clone insect resistance gene belonging to nucleotide binding, leucine rich repeat family of resistance genes (Rossi *et al.*, 1998). Other cloned resistance genes with similar motifs may also possess insect resistance potential.

Perspectives and strategies for improving resistance

The presence of crucial common domains in the *R* genes for resistance against diverse pathogens and pest including bacteria, viruses, fungi, nematodes and insects suggests their common origin and possible unified signal transduction mechanism leading to HR and SAR. With the thorough dissection and understanding of molecular mechanism of host-parasite interaction, signal transduction and defense response it should be possible to engineer non-specific broad spectrum resistance against diverse isolates of different pathogens and pests.

A majority of the transgenic plants expressing any one of the PR genes are incapable of providing the desired level of resistance for commercial exploitation. Combinatorial approach using two or more PR genes of different types through simultaneous or sequential transformation or even pyramiding is more likely to yield the desired results.

There are a few cry, PR, herbicide tolerant and other genes, cloned and modified for adequate expression, which are being used for genetic engineering of all types of plants for disease and insect-pest resistance around the world. The engineered genes for resistance once introduced into a plant behave just like the resident R genes irrespective of their origin and are likely to be overcome by the emergence of new biotypes of the co-evolving pathogen or pest. The search for new genes and combination of active domains of different genes should be pursued vigorously to avoid epidemics. The wild relatives of crop plants could be excellent sources for new R genes with broad spectrum of resistance which have not been transferred due to barriers of sexual hybridization.

Agrobacterium-mediated genetic transformation being routinely used in dicot plants continues to be a distant dream in most of the cereals meeting more than half the calorific requirements of global population (Brar *et al.*, 1996, de Lumen *et al.*, 1997). Cooperative efforts by public and private organizations should be made for efficient and variety-independent *Agrobacterium* transformation in cereals as based on the basic information on plant-bacterium interaction. This would lead to the required transgenic copy control to avoid homology dependent transgene inactivation.

Due to homology dependent transcriptional and translational transgenic inactivation and public concern on biosafety it will not be possible to use the same promoter and selectable markers for repeated transformation of transgenic plants. New vectors based on Cro/lox-mediated system (Vergunst *et al.*, 1998) or MAT system (Ebinuma *et al.*, 1997) may bypass such constrains.

Reduced expression of native bacterial genes and promoters and gene flow to weedy and wild relatives of plants are a matter of great concern for scientists and ecologists. Protocols for routine transformation of chloroplasts will be extremely useful for efficient engineering of plants for resistance and physiological traits.

Negotiated exchange of transgenic technology to the developing countries at easy terms and its integration with the conventional approaches for resistance breeding will ensure evergreen revolution crucial for global food security.

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