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Mini-Review

Molecular Breeding for Plant Disease Resistance: Prospects and Problems

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The technique of plant transformation has started to show off its great power in the area of plant breeding by commercially successful introduction of transgenic varieties such as herbicide tolerant soybean and insect resistant corn in USA with an unimaginable speed. However, in contrast with the great success in the commercialization of herbicide tolerance and insect resistance, the transformation works on disease resistance has not yet reached the stage of full commercialization. This review surveys the current status of molecular breeding for plant disease resistance and their limits and problems. Some novel ideas and approaches in molecular breeding for disease resistance are introduced.

Keywords **:** disease resistance, molecular breeding, transformation, transgenic varieties.

We are experiencing an exciting period in breeding for disease resistant crops. Although conventional plant breeding has made a significant impact by improving the resistance of many crops to important diseases, it became more difficult to develop new cultivars resistant to ever-changing pathogens by the conventional plant breeding because natural resources of resistance available to breeders have been quickly exhausted. As an effective alternative, many plant breeders who are interested in the development of durable and wide-spectrum disease resistant varieties have paid a great attention on molecular breeding. The advent of plant transformation and advanced molecular techniques for plant breeding provides powerful tools for genetically improving crops so as to enhance their resistance to diseases. The distinct advantage of transgenic technology is that it enables the plant breeder to cross species barriers, allowing genes from non-related plants and other organisms be introduced into crop plants.

Plant Biotechnology Setting up Historical Record in Plant Breeding History

It appears that the development of GM (genetically modified) varieties are one of the most successful stories in plant breeding history, since it took only four years for the Roundup Ready Soybean (herbicide tolerant) to occupy more than half of the total acreage of soybean in USA. So far, the most successful story was the development of hybrid corn, but it took seven years for selected States such as Iowa, Illinois and Wisconsin to reach the similar adoption levels. In some other States it took more than twenty years. *Bacillus thuringiensis* corn (Bt-corn), Roundup Ready- and Bt-cotton also exhibit adoption rates significantly faster than hybrid corn (Kalaitzandonakes, 1999a). Hillyer (1999) commented that biotechnology seems to add another chapter to the revolutionary changes that have shaped U.S. agriculture over the past 100 years. Just like the switch from horses to horsepower and mechanical weed control to chemical control, genetic engineering will forever change how farmers produce crops. Also he said that unlike previous breakthroughs, biotechnology may rewrite the book on production agriculture and the entire industry.

Global acreage of GMOs increased tremendously as 1.2 million ha in 1995, 2.8 million ha in 1996, 12.8 million ha in 1997, 27.8 million ha in 1998 and finally 39.9 million ha in 1999. The countries listed in descending order of transgenic crops area on a global basis in 1999 are: USA with 28.7 million ha (72% of the global area), Argentina with 6.7 million ha (17%), Canada 4.0 million ha (10%) and China 1.0 million ha (1%). Transgenic soybean and corn continued to be ranked first (21.6 million ha, 54%) and second (11.1 million ha, 28%) in 1999, respectively. Third is cotton with 3.7 million ha (9%) and fourth canola with 3.4 million ha (9%). The relative ranking of the principal transgenic traits in 1999 was as follows; herbicide tolerance being far the highest (28.1 million ha, 71%), and insect resistant GMOs being the second (8.9 million ha, 22%), and stacked genes for insect resistance and herbicide tolerance being the third (2.9 million ha, 7%). Surprisingly, virus-resistant GMOs were planted less than 0.1 million ha over the world (James, 1999).

There are two major questions to be raised on GMOs production agriculture. First, what are the factors driving the such speedy adoption and diffusion of bioengineered crops on the farm? Second, what are the economic benefits delivered by such technologies, and how have such benefits

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been shared between the farmers and the innovators? For the first question, Carpenter and Gianessi (1999) presented that while some potential cost savings may have driven adoption, the primary reason American growers are switching to new programs like as Roundup Ready Soybean is the simplicity and flexibility of a weed control program. It was very timely that American soybean growers have been looking for varieties such as Roundup Ready Soybean, which will be fitted to on-going trends of cultivation towards postemergence weed control, adoption of conservation tillage practices (i.e. notillage) and narrow row spacing. For the second question, there is no complete reports of economic analyses on GMOs yet. In a study of Traxler and Falck-Zepeda (1999) on the case of *Bacillus thuringiensis* (Bt) cotton, they attempted to partition the benefits among United States domestic and rest of the world consumers of cotton lint, the companies developing the Bt-cotton, and U.S. cotton farmers by introducing Bt-cotton in the United States. The benefit calculations were based on comparisons of pest control costs and yields for Bt- and conventional cotton varieties. They estimated that an average of more than $200 million per year in benefits were generated by the use of Bt-cotton. In each year, U.S. farmers received the largest single share of benefits, ranging from 42% to 59% of total surplus generated. The combined share of Monsanto and the seed firms ranged from 26% to 44%. It is clearly indicating that at present American farmers are those who are getting major portion of benefits generated from plant biotechnology, which makes the American farmers who already have a strong competitive power at the international market become even much stronger.

Now, there are very serious questions to be raised for the future of biotechnology in less developed countries (LDCs). Can less LDCs benefit from agrobiotechnology? If LDCs can indeed benefit from agrobiotechnology, what are the likely pathways of implementation (e.g. develop indigenous technology, transfer technology, and trade for products)? Should strategies and priorities in agrobiotechnology in LDCs be different from those of U.S.? Then, how much and how can it be done? It seems that almost everyone who is doing researches on biotechnology in LDCs is busy thoughtlessly to copy or mimic what American counterparts have been doing for the last decade. Since each country differs in stage of development and has her own unique practices and constrains in their agriculture, no one should be sure that the successful story of agrobiotechnology realized during the last five years in US can be possibly duplicated in LDCs also. There are some good references on pros and cons for issue of agrobiotechnology in the LDCs (Altieri and Rosset, 1999; Kalaitzandonakes, 1999b; McGloughlin, 1999; Ruttan, 1999).

It is thought to be the right time that plant biotechnologists and plant breeders in Korea together should try to set up our own priority and strategy in utilization and application of biotechnology, which should be most suitable and workable for Korean conditions. GMOs such as herbicide tolerant and Bt varieties which are getting immense popularity in U.S. may not be well fit into the Korean agriculture system, mainly because of small farm size and less mechanized farming in Korea. Instead, disease resistant GMOs will be most needed for agriculture in Korea, if multiple and durable disease resistance varieties could be developed.

Poor Commercialization of Disease-resistant Transgenic Varieties

Whatever Happened To All of Those Researches?

It is very disappointing to notice that the rate of commercialization of disease resistant GMOs are extremely low as being only less than 0.1 million ha (0.25% of total area under GMOs) out of a total of 39.9 million ha in 1999 globally. It would be impossible to estimate exact amount of R&D spent on development of disease resistant GMOs. But, we can roughly guess that about 20% of total biotechnological R&D in America were drained on molecular breeding for disease resistance, because numbers of field trial permits issued to disease resistance GMOs are about 21% of the total (Table 1). For the last 13 years in America alone, about 1,085 viral resistant, 431 fungal resistant and 84 bacterial resistant GMOs may have been tested in fields. The reason why these disease resistant GMOs have not been widely planted at farmers' field remained without any clear-cut explanation at least in the literatures which I surveyed. I guess farmers want to have very high degree of resistance (something like immune), durability and broad-spectrum disease resistance (i.e. nonhost resistance).

Since one of our main objectives of the center (PMGB) is to develop techniques and/or genotypes related with plant disease resistance, first we have to find out the reasons of relatively poor performances of disease resistant GMOs in commercialization of developed countries, where molecular breeding for disease resistance has been researched much earlier and also much deeper in depth. It is strongly recommendable that we should develop our own research strategies and priorities in molecular breeding for disease resistance, which must be suitable to our farming situation.

Current Status of Molecular Breeding For Viral Resistance

Since the first transgenic virus-resistant plants to tobacco virus using its coat protein gene were produced in 1986, numerous transformations have been conducted with fairly good success. Many of those virus-resistant transgenic vari-

Table 1. Numbers of field trial permits of GMOs by traits issued by USDA during 1987- June 2000 and their corresponding acreage in global adoption

Traits	Numbers of field trial permits issued by USDA (1987-June, 2000)		Worldwide Acreage of GMO's cultivated at farmers field (1999)	
	Number	%	Acreage (M ha)	%
Herbicide tolerance	1,973	26.1	28.1	71
Insect resistance	1,725	22.8	8.9	22
Herbicide tolerance + Insect resistance			2.9	7
Product quality	1,292	17.1	–	–
Viral resistance	1,085	14.4	0.1	0.25
Agronomic properties	436	5.8	–	–
Fungal resistance	431	5.7	–	–
Marker genes	241	3.2	–	–
Bacterial resistance	84	1.1	–	–
Nematodal resistance	9	0.1	–	–
Others	274	3.6	–	–
Total	7,550	100	3,990	100

eties were tested in open-field, confirming not only the durability of the resistance under natural conditions but retaining of original cultivar traits. In America alone, more than thousand viral-resistant GMOs were permitted to be tested in the field. However, as mentioned earlier, actual acreage under these viral-resistant GM varieties were only 0.1 million ha out of a total 39.9 million ha for whole GMOs in 1999. The reasons why the viral-resistant GMOs have not been accepted by American farmers are not known in this part of the world (Kavanagh and Spillane, 1995).

A number of alternative anti-viral strategies based on transgenes from a surprisingly wide variety of sources have been developed. These include the use of viral genes coding for proteins involved in the replication cycle and in system transport of viruses within the plant, the use of interfering viral RNA sequences, and the use of transgenes derived from plant and animal sources. In the latter category, the use of mammalian antibodies to confer disease resistance in plants is particularly new exciting development. Considerable progress has also been made towards the molecular cloning of natural anti-viral resistant genes in plants.

Now, there are numerous reports on gene silencing available. Waterhouse et al. (1999) reported that virus-derived transgenes in plants can be poorly expressed and yet provide excellent virus resistance, and transgene constructs designed to supplement the expression of endogenous genes can have the effect of co-suppressing themselves and the endogenous genes. These two phenomena appear to result from the same post-transcriptional silencing mechanisms, which operates by targeted-RNA degradation. Recent research into RNA-mediated virus resistance and co-suppression has provided an insight into the interactions between plant viruses and their hosts, and spawned several modes to explain the phenomenon. They also reported that PTGS (post-transcriptional gene silencing) is a powerful, specific, and intracellular RNA-degradation mechanism, which has probably evolved as defence against virus infection. In spite of intensive researches over recent years, the components of the mechanism remain largely unknown. The imminent completion of the *Arabidopsis* genome sequencing project, in combination with PTGS mutants in *Arabidopsis*, might also identify the genes involved in PTGS and its mechanism too.

Current Status of Molecular Breeding for Fungal Resistance

It has to be clearly pointed out that the progress in commercialization of fungal resistant transgenic varieties has been far behind compared with insect- and virus-resistant GM. Some experts are now anticipating that it will take another 4-8 years to have successful commercial introduction of fungus-resistant crops at farmers' field in the developed countries such as U.S.A. (Honee, 1999; Melchers and Stuiver, 2000). It means that it may take much longer in LDCs including Korea to have commercial GMOs for resistance to fungal diseases at farmers' field. The main reason for this delay is that markets (i.e. farmers) want durable and broad-spectrum disease-resistant GM varieties, not GMOs with just only a single transgene available.

Now there are two major approaches in developing durable and broad-spectrum fungus-resistant GM varieties. The first strategy relies on the expression of genes that encode proteins which have either direct or indirect inhibitory effects on the growth of fungi (namely, the antifungal pro-

tein strategy). The second strategy aims to increase fungal resistance in plants by transferring the system of hypersensitive response (HR), which needs specific interaction of the product of the avirulence (*Avr*) gene and that of resistance (*R*) gene (namly, *Avr/R* two-component strategy) (Melchers and Stuiver, 2000).

Antifungal Proteins Strategy. This strategy involves the constitutive expression of genes encoding antifungal proteins in transgenic plants. It becomes a common practice to introduce two PR genes together into a crop plant, since highly synergistic interactions between the two enzymes in vivo were observed. For example, the constitutive coexpression of tobacco chitinase and β-1,3-glucanase genes in tomato transformants expressed higher levels of resistance to *Fusarium oxysporum* than either gene alone, and simultaneous expression of a rice chitinase and an alfalfa glucanase gene in tobacco also yielded stronger resistance to a fungal pathogen. Similar attempts which were tried to combine three genes encoding as class II chitinase, class II glucanase and type I ribosome-inactivating protein (RIP), respectively, from barley to transgenic tobacco showed stronger resistance. This strategy was also successful recently in monocot crops, including rice and wheat, in developing resistance to *Rhizoctonia solani* and *Fusarium* species.

In addition to PR protein, there are some antifungal peptides such as defensin, thioins, hevein and lipid transfer proteins, which were used as transgenes in developing stronger resistance varieties. For example, one of plant defensin genes (such as Rs-AFP2 from radish encoding a small cysteine-rich defensin) was transformed to tobacco, resulting in a high level of resistance to *Alternaria longipes*. Interestingly, the induction of the endogenous genes for these antifungal peptide after pathogen attack is independent of PR-gene expression, suggesting that these defenses are activated through independent pathways. The constitutive expression of H_2O_2-generating enzyme, glucose oxidase in potato, provided disease resistance to a range of plant pathogens including *Erwinia carotovora, Phytophthora infestans* and the *Verticillium* wilt pathogen.

After thoroughly reviewing transformation experiments using antifungal proteins, Melchers and Stuiver (2000) suggested that future studies will be necessary to identify combinations of defense proteins that might confer even more effective broad-spectrum protection.

Hypersensitive-Reponse-Based Stategies. The hypersensitive response (HR) is one of the most powerful and remarkable self-defence mechanisms against pathogen attack, which plants have been developing through a long period of coevolution. Currently the most interesting research related with GMOs utilizing HR is to design HR for combating a broad-spectrum of plant pathogen. De Wit proposed that the transfer of an avirulence gene (i.e. the *Cladosporium fulvum avr9* gene) into a plant containing the corresponding resistance gene (i.e. the tomato *Cf9* gene), and its subsequent expression under the direction of a promoter that is rapidly and locally inducible by a wide range of fungal pathogens. Using this technology Melchers and Stuiver (2000) were able to develop new tomato GMOs with increased resistance to a broad-spectrum of diseases including multiple fungi with different infection modes and also viruses. With the continuing elucidation of the signal transduction pathways involved in plant defense responses, more and more molecular components are becoming available that can be used as tools to induce the HR either completely or partially. Also further understanding of molecular mechanism responsible for the resistance mediated by the Avr-R genes will be critically utilized in establishing durable resistance in genetically engineered crops. Futhermore, tightly regulated promoters are needed that are induced exclusively upon pathogen attack. (Honee, 1999).

Current Status of Molecular Breeding for Bacterial Resistance

It is generally believed that molecular breeding for bacterial resistance is far more difficult compared with that for viral and fungal resistances. It will take sometime to get commercial GMOs which have durable and broad-spectrum disease resistance to bacterial pathogens. In the case of bacterial pathogens, the molecular study of genes and mechanisms of pathogenesis and natural or induced plant resistance, and parallel work with antibacterial protein from various sources, have provided a basis for implementing a range of molecular breeding strategies to introduce novel forms of transgenic resistant plants. These approaches fall in three basic categories: (i) introduction of bacterial avirulence genes; (ii) incorporation of pathogen-derived genes for resistance to bacterial phytotoxins; and (iii) expression of antibacterial proteins from plants, insects or bacteriophages as bactericidal or bacteriolytic agents (Panopoulos et al. 1996). The engineering of resistance to bacterial disease by molecular breeding potentially can tap many different sorts of genes, derived from the pathogens themselves, the plant they infect, and many other organisms.

Some Critical Review on Flor's Gene-for-gene Concept

Gabriel (1999) seriously criticized Flor's gene-for-gene hypothesis on the nature of 'virulence' allele. In the gene-for-gene hypothesis, Flor proposed the existence of 'pairs of factors' (avirulence and virulence alleles, respectively) in the pathogen in determining the kind of interactions with host plants, namely incompatible (means resistance pheno-

type) and compatible (susceptible phenotype) relationships. Gabriel recognized the existence of avirulence alleles, which are dominant, in pathogen, which likely encode plant cell signal proteins (likely a kind of ligands) that are perceived by receptors (encoded by cognate host R alleles or haplotypes) and triggered signal transduction pathways, finally resulting in various defence systems after through complex cascade reactions.

What Gabriel insisted is that there are no 'virulence allele' existing as Flor proposed in gene-for-gene hypothesis. Flor emphasized the existence of 'virulence allele' and its function was the active determinants of virulence and pathogenicity. More than 40 avirulence genes have been cloned and sequenced to date, and their molecular organizations were clearly shown (Ellis et al., 2000; Richter and Ronald, 2000; Young, 2000). But, none of 'virulence allele' in term of Flor' was found and cloned yet. Gabriel said "it became clear that use of term 'virulence gene' to refer to the hypothetical alternate allele of cultivar-specific *avr* genes was a misnomer; in R gene for *avr* gene interactions, a 'virulence' allele often does not exist, and if it does, does not condition the cultivar-specific state it describes". Unfortunately, there is considerable confusion in the literature brought about by use of the term 'virulence' as synonymous with 'pathogenicity'. The term 'pathogenicity' should be used only when a gene (haplotype) actually condition pathogenicity and host range in noncultivar-specific manner. The term 'virulence' will only be used to refer to cultivar-specific situations. It is very important to understand Gabriel's critics on concept of 'virulence allele', because so many scientists misunderstand that any recessive mutants of *avr* allele would have became automatically 'virulence allele', so that the mutants condition pathogenicity and host range cultivar-specifically. Truth is that the mutants just allow incidence of disease because the host receptors are not able to perceive changed ligand of mutants. To avoid any more confusion on concept of gene-for-gene response, Briggs and Johal (cited from Johal, 1996) proposed that the name 'avirulence' be replaced by 'incompatibility', because products of an avirulence gene behave more like an 'incompatible antigen' than a factor that contributes to pathogen virulence.

Gabriel (1999) also raised a very interesting question such as "Why do pathogens carry avirulence genes?" or "Why are there so many *avr* genes?" The question of why pathogens carry so many *avr* genes which are so harmful for their survival has been a long-lasting enigma. He explained that most *avr* genes are, or once were, pathogenicity (*pth*) genes essential to biotrophic pathogens that determine(d) host range in host-specific manner. At least some of these genes appear to function for pathogenicity by encoding protein signals that are 'injected' into plant cells by the *hrp* system, resulting in programmed cell death. Another explanation for the enigma is that many *avr* genes may be present because of a variety of stochastic events, including horizontal gene transfer, duplication and mutation. There is good reason to believe that many *avr* genes are gratuitously present in a variety of bacterial strains following horizontal transfer from other species.

Ways To Develop Varieties For Broad-spectrum Resistance

It becomes very clear that farmers in either developed countries or LDCs won't plant eagerly the disease resistance GMOs, if they are not securely equipped with durable and broad-spectrum disease resistance. So, the most urgent and important goal in molecular breeding for disease resistance should be the development of durable and broad-spectrum disease resistant GMOs.

Cao et al. (1998) are interested in understanding immunity in plants and the possibility of identifying target genes for engineering long-lasting, broad-spectrum resistance in crops. They report experiments investigating the possibility of generating disease resistance through overexpression of *NPR1* (nonexpresser of PR) genes. They also explored a different approach for generating broad-spectrum disease resistance in plants, instead of exogenous application of SAR-inducing chemicals. They reported that "we succeeded in enhancing the plants' immunity by overexpressing a key regulatory of SAR signaling pathway, *NPR1*. It appears that this approach does not adversely affect the growth or development of the plants, making it an attactive method for controlling plant disease".

Keller et al. (1999) described interesting experiments with transgenic tobacco that harbours a pathogen-inducible promoter-elicitor fusion. Plant resistance to various (hemi)biotrophics and also necrotropic fungi is observed in these transgenic plants. They have developed a strategy for creating novel disease resistance traits whereby transgenic plants respond to infection by virulent pathogen with the production of an elicitor. To this end, they generate transgenic tobacco plants harboring a fusion between the pathogen-inducible tobacco *hsr203J* gene promoter and a *Phytopthora cryptogea* gene encoding the highly active elicitor cryptogin. The transgenic plants displayed rapid HR not only to fungal pathogens of *Phytophthora* species but also to other pathogens unrelated to *Phytophthora* species, such as *Thielaviopsis basicola, Erysiphe cichoracearum,* and *Botrytis cinerea*. Thus, broad-spectrum disease resistance of a plant can be generated without the constitutive synthesis of a transgene product.

Ellis et al. (2000) reported that mutation experiment, in which R-gene signalling has been dissociated from speci-

ficity in constitutive signal mutants, have provided the potential for non-specific resistance to be expressed from pathogen-infection-induced promoters in transgenic plants.

Future Molecular Breeding For Disease Resistance

Michelmore (2000) suggests that we already have unimagined tools and capabilities in pursuit of molecular breeding for disease resistance compared with those available a few years ago. He considers that sequence comparisons and functional analysis will allow dissection of the molecular basis of specificity; and this in turn will lead to the *ex planta* generation of new resistance gene specificities, and also genomic approaches are beginning to revolutionize our understanding of plant diseases resistance. Large-scale sequencing will reveal the detailed organization of resistance-gene clusters and the genetic mechanisms involved in generating new resistance specificities. Global functional analyses will elucidate the complex regulatory networks and the diversity of proteins involved in resistance and susceptibility. He concluded that in the future, resistance genes will be designed and synthesised at laboratory that recognize essential components of pathogens and then induce the appropriate response pathways, which will make plant breeders be able to develop durable and wide-spectral disease resistance.

Ellis et al. (1999) also suggested that the ability to copy the evolutionary process of generation of new disease resistance specificities by genetic engineering may provide novel specificities for control of plant disease in agriculture.

Cary et al. (2000) suggested to use synthetic antimicrobial peptide as transgenes in molecular breeding for disease resistance. Antimicrobial peptides are excellent candidates to augment disease resistance mechanisms in plants due to their (i) rapid biocidal or biostatic ability; (ii) activity against a wide spectrum of organisms at low concentrations; and (iii) nontoxic nature with respect to mammalian cells. The advent of automated peptide synthesizers and combinatorial peptide chemistry has made it possible to rapidly synthesize, and screen large numbers of peptides for their ability to inhibit the growth of target microbial pathogens. These linear peptides often can be less than half the size (10-20 amino acids) of their native counterparts and many times more potent without concomitant toxicity to host issues. DeLucca et al. (cited from Ellis et al. 2000) reported on the antifungal activity of a 17 amino acid linear, synthetic peptide designated D4E1. This peptide was shown to interact with sterols present in the conidial cell walls and resist degradation by fungal and host proteases.

Revers et al. (1999) reported that a recent exciting development in our understanding of viral pathogenesis is that HC-Pro (helper component-proteinase) may act as a general pathogenicity enhancer by interfering with a host defense response that normally limits viral infection. Several researches have shown that HC-Pro has the capacity to suppress PTGS (post-transcriptional gene silencing), and that the consequence of this enhanced virus replication. They concluded that major challenge for the future will be take advantage of the new opportunities offered by chip-based expression arrays, computer-assisted functional genomics analyses, an the rapidly accumulating knowledge of the host genome (particularly *A. thaliana*) to develop advanced studies of both fundamental and applied areas of potyviral biology.

Simpson et al. (2000) reported the complete genome sequence of *Xylella fastidiosa*, causal bacterial pathogen of citrus variegated chlorosis. The genome comprises a 52.7% GC-rich 2,679,305-bp circular chromosome and two plastids of 51,158 bp and 1,285 bp. They can assign putative functions of 47% of 2,904 predicted coding regions. They reported that the mechanisms associated with pathogenicity and virulence such as toxins, antibiotics and ion sequestration systems become more clearly understood through the genomic analyses. The most interesting is that at least 83 genes are originated from other bacteria by horizontal gene transfer such as bacteriophage-derived conjugation, which is the first report showing the direct evidence of horizontal gene transfer in evolution of pathogenic bacteria. Indeed, the availability of this first complete plant pathogen genome sequence will now allow the initiation of detailed comparison of animal and plant pathogens at the whole-genome level. In addition, the information contained in the sequence should provide essential aspect of interaction between *Xylella fastidiosa* and its hosts that might lead to fresh insight into potential approaches to control (of course including molecular breeding for disease resistance) of citrus variegated chlorosis virus. We, plant breeders, are very anxious for waiting more information from whole genomic sequence analyses on either plant hosts or pathogens, which will be poured out in the near future.

Conclusions

Molecular breeding for disease resistance is entering an exciting period during which numerous new tools (as some samples; large-scale genome sequencing, re-sequencing of R gene clusters, the use of degenerate PCR primers to harvest R-gene candidate, comparative genomics and phylogenetic analyses) are available, and understanding of defence mechanism, pathogenicity, evolution of R gene especially related with mechanism of generating new disease resistance specificities, detailed structure of R genes (i.e. clustered arrangement). All of informations and tools will be utilized in development of disease resistance GM varieties

having durable and broad-spectrum resistance, which will be gladly accepted by farmers both in developed countries and LDCs hopefully in the near future.

Plant breeder, who is interested in molecular breeding for disease resistance, should be well prepared for the future, in which paradigms, concepts, procedures and techniques in plant breeding will be quite different from what we have been familiar with for a long time.

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