SCOPE OF TRANSGENIC APPROACH FOR PLANT DISEASE RESISTANCE

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Abstract: Transgenic plants or genetically engineered plants have foreign DNA introduced in them and foreign DNA integrates with the host plant DNA to express new trait. Therefore, transgenic plants in food, fibre, vegetable and fruit crops have led to improved crop yields. For transformation of genes, irrelevant organisms, bacteria and viruses are used. Though, the success of transformation is limited however, the concept has wider scope of applications and this is growing continuously. Earlier, monocot species were not considered as host range of *Agrobacterium tumifaciens*, which led to the development of direct DNA introduction. However, recently Agrobacterium mediated transfer has been reported in monocot species including the most important food crops like rice, maize and wheat for various traits. Genetic transformation has led to the possibility of transforming crops for enhanced resistance to insects and diseases caused by fungus, bacteria and viruses. In the past knowledge of understanding plant-pathogen interactions in molecular terms has led to the identification of disease resistant plant genes that specify race-specific resistance to pathogens. Incorporation into the plant of a gene that encodes the coat protein of the virus protects it from disease and this approach can be applied in crops facing viral infestation such as cotton leaf curl disease (CLCuD) and *Pto* gene transfer in tomato plant against bacterial infection showed some scope to cope with the continuously occurring disease problems. More progress in development of disease resistant transgenic plants will be seen in the near future. Efforts are required to explore this approach for plant disease resistance with knowledge of specific gene functions and potential side effects.

Keywords: Transgenic, DNA and plant diseases.

Introduction: Plants survive in nature with their genetic strength and their potential is always challenged by various biotic and abiotic stresses. Among biotic stress role of disease always have been very significant back in the history. Famine due to diseases in plants affected human survival worldwide at various times. Resistance in plants against many diseases has been always a challenge to plant pathologists and the farmer community that faces consequences of disease epiphytotic in term of economic losses. With the time advanced technologies paved new ways to cope with plant diseases with new methods and new principals. Use of biotechnology is very much common to apply in agriculture in various fields. History of transgenic is not new to human being, however, the exploitation of this technique has created wider scope for its application to control plant diseases.

Importance of transgenic against plant diseases: Studies showed that many crops showed susceptibility to multiple diseases caused by fungus, bacteria or viruses and bear serious economic losses. In addition, infected plant material grown for export purpose may also face rejection at international level (Loebenstein *et al*., 1995). These pathogens have been the major constraints that are very difficult and sometime impossible to control once it is inserted within the plant body. Therefore, direct and indirect effects of plant pathogens and infections include reduction in growth, reduction in vigor, costs of attempting to maintain crop health & reduction in quality and market value of agriculture produce (Hadidi *et al*., 1998).

Approaches for induction of disease resistance in plants: The approach of genetic engineering offers new ways to control diseases in plants. The main approaches to incite disease resistance includes:

1. Introduction of new genes that encodes for specific enzymes such as chitinase (breakdown chitin), glucanase (breakdown glucan) in plants to control fungal diseases by disrupting fungal cell wall.
2. Some of the innate plant defense mechanisms such as production of phytoalexins, PR (pathogenesis related) proteins, toxic protein production can be triggered in plants by the introduction of genes in to the plant cells.
3. Introduction of gene that promotes hypersensitive response in the plant which is major character of most of the resistant varieties against specific race of the pathogens.

Methods and techniques in transgenic:

Genetically modifying a plant with *Agrobacterium tumefaciens*: Among the number of techniques used for GM plants, the two most commonly employed are the bacterium *Agrobacterium tumefaciens*, which is naturally able to transfer DNA to plants and the 'gene gun', used to shoot DNA coated microscopic particles within plant cell (Southgate *et al*., 1995). Generally, individual plant cells are targeted and these are regenerated into whole GM plants using tissue culture techniques. Use of *A. tumifaciens* has become very much common in agriculture and has been exploited at various levels. *Agrobacterium* mediated transformation has its very important position to cause successful transformation in plants for various characters. Researchers are enjoying the ability of *Agrobacterium* in transferring different gene responsible for various characters. Genes responsible for disease resistance can be transferred in to widely grown variety that has high yield potential but is susceptible to disease and this would cause breeder to change the variety with lesser yield potential sometime. Successful application of this technique is seen in development of *Bt*-cotton in India. Bt (Bacillus thuringiensis) is ubiquitous bacterium and have property to produce crystal like protein that may cause cell lysis in insect body and leads to death of the insect. The new varieties with *Bt* gene that have insecticidal activity are developed by using *Agrobacterium* mediated transformation and later by applying traditional breeding approaches that gene is transferred to various cotton hybrids for particular areas.

The procedure used in transformation has put researchers in discussion about its effect on human health. Three procedures are:

1. The use of selectable markers to identify transformed cells
2. Transfer of extraneous DNA into the plant genome (i.e. genes other than those being studied)
3. The possibility of increased mutations in GM plants compared to non-GM counterparts due to tissue culture processes used in their production and the rearrangement of DNA around the insertion site of foreign genes.

To facilitate the transformation process, a selectable marker gene conferring, for example, resistance to an antibiotic (e.g. kanamycin, which will kill a normal non-GM plant cell), is often co-transferred with the gene of interest to allow discrimination of GM tissue and regeneration of GM plants. However, these genes were initially isolated and are already widespread in the bacterial population. In addition, kanamycin itself generally regarded as safe and has been used for over 13 years without any known problems.

Use of promoters: Up to now, evident increase in disease resistance has been obtained by many attempts at engineering and used constitutive over-expression of the transgene. In the past only dicots plants were transformed successfully whereas, monocots were not possible to transform with foreign genes. Recent advances in technology and research made it easy in monocots also. The CaMV 35S promoter has been commonly used in dicotyledonous plants, whereas other promoters such as the maize ubiquitin promoter have been used in monocotyledons (Gurr and Rushton, 2005). The choice of these promoters has often led to problems. Constitutive overexpression of defence components can lead to increased disease resistance but it often comes at a price. The plants might have reduced size (Chen and Chen, 2002), altered morphology (Li *et al*., 2004) or they show disease symptoms in the absence of pathogens (Fizgerald *et al*., 2004).

***R*-genes:** Researchers have gained deep knowledge of understanding race-specific pathogen resistance controlled by single dominant resistance (*R*) genes. These gene can be cloned in large number that shows *R*-gene products and share motifs such as leucine-rich repeats (LRRs), nucleotide binding sites and kinase domains. They have their unique role in signaling pathways and is responsible for one the mechanisms for pathogen recognition which might be exploited for applied goals (Hammond-Kosack and Jones, 1997). It is observed that R-genes shows instability in the field and some *R*-genes exhibit a broader spectrum of activity against a given pathogen species. For example, the *Xa21* gene from rice exhibits activity against 29 distinct races of the bacterial blight pathogen *Xanthomonas oryzae* (Wang *et al*., 1996).

Gene silencing: RNA silencing-based resistance has been a powerful tool that has been used to engineer resistant crops. Based on this mechanism, diverse approaches were developed with the time. In gene silencing an endogenous gene is suppressed by introduction of related transgene used for crop improvement. Viruses are potentially both initiators and targets of gene silencing suggested that this phenomenon may be related to natural defense against viruses. By using this phenomenon nepovirus infection of non transgenic plants can induce resistance mechanism similar to transgene-induced gene silencing (Ratcliff *et al*., 1997). Virus induced gene silencing can be used to access the gene function of different plants cDNA in disease resistance. cDNA was used in *Pto* mediated resistance against *Pseudomonas syringe* in *Nicotiana benthamiana*. The loss of resistance was also observed in plants in which cDNA suppressed hypersensitive response in plants (Lu *et al*., 2003). They also found that there is one gene product HSP90 protein that has its role in disease resistance with its role in plant growth and development.

By use of sense and antisense viral sequences in transgene-mediated phenomenon, resistance can be employed in plants. From the old studies on Tobacco mosaic virus & Citrus tristeza virus it was believed that viral proteins expressed from the transgenes conferred resistance (Abel *et al*., 1986 and Fagoaga *et al*., 2006) to later studies conferred that that plants expressing the truncated viral protein sense sequence or the non-coding viral sense sequence, such as the satellite RNA sequence (Harrison *et al*., 1987 and Fagoaga *et al*., 2006), also showed disease resistance to some extent. Furthermore, plants expressing antisense viral sequences also conferred high resistance. The findings imply that the RNA sequence is also involved in resistance induction and this suggests involvement of some type of novel mechanisms which is sense transgene-induced in nature (Beclin *et al*., 2002).

Conclusion: Plant always face biotic and abiotic stresses with changing environmental conditions and develop their niche for their survival. However, the crops grown over a larger area are exposed to more biotic and abiotic stress conditions. Resistance to different types of stress conditions become a challenge for breeders to develop varieties that can sustain for longer under variable pressures of stress. A crop plant faces new race developments of pathogens that break resistance in crop. Biotechnology has its own beauty to go deep in to the molecular level and can be used to alter genetic makeup of plants for specific character/s. the plants developed with genetic engineering have foreign DNA introduced in them and foreign DNA integrates with the host plant DNA to express new trait. It leads to improvement in crop yield characters as well as disease resistance against the pathogens. By using *Agrobacterium tumifaciens*, promoter genes, *R*-gene and gene silencing desired characters can be induced in to the plant. With the identification of particular genes responsible for disease resistance for specific race of pathogen, the resistance can be developed with the incorporation of that gene by using genetic engineering techniques. Genetically transformed crops show resistance to insects and diseases caused by fungus, bacteria and viruses. This field of advance technology has brighter future and would have broad application in near future for crop advancement and inducing resistance in crops for economically important diseases.

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