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Review Article

GENETIC ENGINEERING FOR IMPARTING ABIOTIC STRESS TOLERANCE IN RICE - A REVIEW

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Abstract- Rice is a very important cereal crop, grown on 160 million hectares worldwide. It is a chief and primary resource of pabulum for more than a moiety of the population and more than 90 per cent of the world's rice is grown and consumed in Asia. Abiotic stress conditions such as cold, salinity and drought are the major limitations in modern agriculture, which negatively influence plant growth and productivity. Hence, it is high time to develop crop plants with traits conferring tolerance to these stresses. Conventional breeding and marker assisted breeding have substantially contributed to our thoughtful of the complexity of stress response. Whereas, genetic engineering techniques provide an efficient and faster way to insert beneficial genes of interest originating from not only same species but also cross barrier species and distant relative sources. *Agrobacterium*-mediated transformation and biolistic transformation are the most common methods used to introduce the gene enclose into plant cells. This review explains about the basic steps of Agrobacterium-mediated transformation for abiotic stress tolerant rice.

Keywords- Abiotic stress tolerance, Rice, Genetic engineering, *Agrobacterium*, Gene transformation.

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Introduction

Rice is fortifying the life of preponderant number of people living in Asia since it was domesticated between 8,000 and 10,000 years ago. It is a source of income for more than 100 million householders around the world [1]. Rice is a very important food of nearly half the world's population and is main crop cultivated by more than 50% of world's farmers, and the most widely consumed staple pabulum crop and is grown on 160 million hectares ecumenical [2]. This crop has shaped the cultural, gregarious, and economic development in Asia. Rice is critical for survival of astronomically immense population. Though the rice plant is mostly dihydrogen monoxide doting, it is adopted to and grown in virtually all ecologies such as irrigated, upland, low land, submerged, and hilly conditions. However, its engenderment is often perturbed by several abiotic factors such as water, high salt, and temperatures. Nearly 22 % of the agricultural land is saline globally [3].

Due to intricate polygenic nature of abiotic stress tolerance, efforts to progress this trait in desirable cultivars by conventional and marker assisted breeding methods have met with little accomplishments. Alternatively, the identification and transfer of favorable genes through transgenic tools are often estimated as one solution for fending crops against dehydrogenate monoxide stress environment and incrementing crop yields ecumenical, categorically in less developed areas that are threatened by victuals scarcity and low crop productivity [4]. The transgenic tactic comprises structurally altering characters by transmitting chosen genes from one species to another [5] and it has been employed to over express genes from the model dicotyledonous plant *Arabidopsis* in many crop plants and vice-versa.

The population is rising at a startling rate and is projected to stretch about six billion by the end of the year 2050 [6]. On the other hand, agricultural productivity is not incrementing at the required rate to keep up with the victuals demand because of water scarcities, exhausting soil potency and several abiotic stresses.

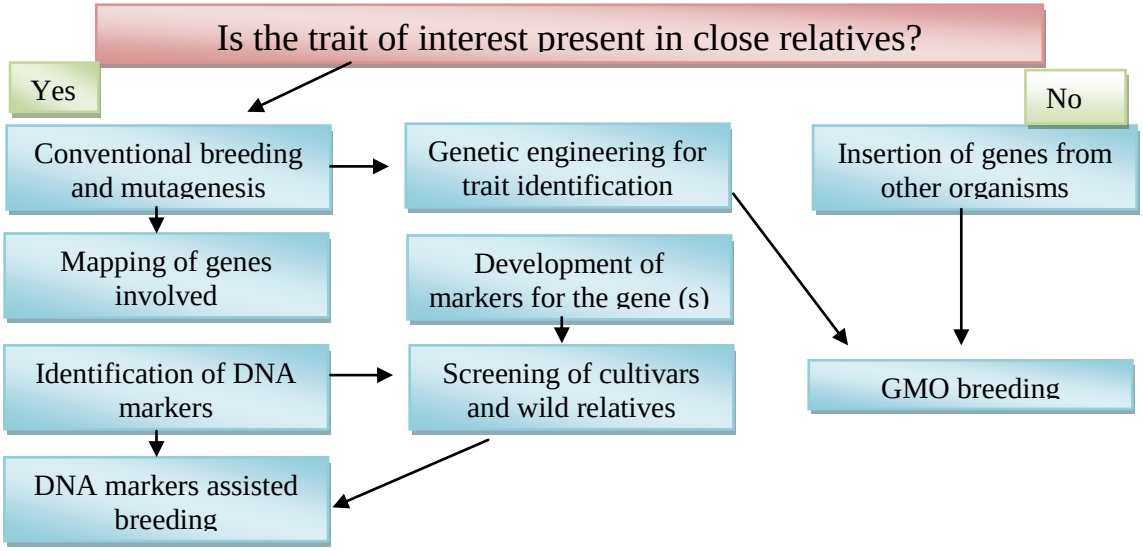
Stress is defined as any environmental variable, which can induce a potentially injurious strain in plants. Abiotic stresses adversely affect magnification and productivity and trigger a series of morphological, physiological, biochemical and molecular vicissitudes in plants. Submergence, drought, saline soils and temperature extremes are the most mundane stresses plants come across. Ecumenically, approximately 22% of the agricultural land is saline [2]. The major abiotic stresses (drought, high salinity, algid, and heat) negatively influence the survival, biomass engenderment and yields of staple aliment crops up to 70% [7-9]. Ergo, minimizing these losses is a major area of concern for all nations to cope with the incrementing victuals requisites. The concept of "optimal magnification conditions" is a fundamental principle in biology. Since living organisms cannot control environmental conditions, they have evolved two major strategies for surviving adverse environmental conditions, i.e., Stress avoidance, or Stress tolerance

The avoidance mechanism is most conspicuous in warm-blooded animals that simply move away from the region of stressful stimuli. Plants evolved intricate biochemical, molecular and genetic mechanisms to eschew stress. On the other hand, tolerance mechanisms mainly involve biochemical and metabolic designates which are in turn regulated by genes. Some of these genes are categorical for a particular stress while others are shared between sundry stresses. Abiotic stress triggers a number corresponding genes, which alter the levels of numerous proteins and metabolites, which may provide a certain degree of tolerance to these stresses. The vital point for breeding and developing better crops to tolerate and survive stress has always been to fathom the vagaries in cellular, molecular and biochemical machinery that follow in replication to stress. Modern molecular techniques involve the identification and utilization of molecular

markers that can enhance breeding programs. The development of genetically engineered plants by the exordium and/or over expression of culled genes seems to be a viable option to hasten the breeding of "improved" plants. Intuitively, genetic engineering would be a more expeditious way to insert benign genes than through conventional or molecular breeding. Withal, it would be the single route when genes of concern coin from cross barrier species, distant relatives or from non-plant sources. Indeed, there are numerous characters whose correlative sodality with resistance has been confirmed in transgenic plants. Following these logical steps, sundry transgenic technologies have been used to ameliorate stress tolerance in plants [10]. Genetic transformation has sanctioned the prelude of incipient pathways for the biosynthesis of sundry compatible solutes into plants, resulting in the engenderment of transgenic plants with amended tolerance to stress [11].

Genetic Engineering

Genetic engineering in crop is becoming more and more widespread. In 2005 an estimated 222 million acre of GE crops were grown by 8.5 million growers throughout 21 nations [8]. Genetic engineering systems are exploited only when all other methods have been drained, i.e. when the characteristic to be introduced is not extant in the germplasm of the crop; the trait is very grueling to enhance and when it is time consuming to amend such trait in the crop by conventional breeding approaches [Fig-1]. Crops advanced through genetic engineering are usually kenned as transgenic crops or genetically modified (GM) crop.



Source: DANIDA, 2002 [12]

Fig-1 Comparison between conventional and modern breeding method

How to Develop Transgenic Plants?

Albeit there are many diverse and in volute techniques involved in genetic engineering, its fundamental principles are plausibly simple. There are five key steps for genetically engineered crop development, which are very important to ken the physiological, and biochemical mechanisms of action and regulation of gene expression as well as safety of the gene and gene product to be utilized. For commercial use, the genetically engineered crops also have to pass through rigorous safety and valuation trials.

1. The first step is the extraction of DNA from the organism known to have the trait of interest.
2. The second step is gene cloning, which will isolate the gene of interest from the entire extracted DNA, followed by mass-production of the cloned gene in a host cell.
3. Once it is cloned, the gene of interest is designed and packaged so that it can be controlled and properly expressed once inside the host plant.
4. The modified gene will then be mass-produced in a host cell in order to make thousands of copies.
5. When the gene package is ready, it can then be introduced into the cells of the plant being modified through a process called transformation.

The techniques for the transfer of DNA into organisms differ from organism to organisms. Generally there are two approaches for DNA transfer. In first case the transfer of DNA take place by natural method and in second case the transfer is by artificial method.

The cull of systems of DNA transmission rest on the target cells in which transformation will be executed. It furthermore relies on the purposes of gene manipulation. The transfection may be either stable or transient. Albeit, cull of DNA transfer method is very consequential, the other consequential steps are cull of gene, isolation of gene, preparing recombinant DNA and cull of transformed cells. The regeneration of organism with incipient characteristics is withal equipollent consequential. The most prevalent methods used to introduce the gene package into plant cells include biolistic transformation (utilizing a gene gun) or *Agrobacterium*-mediated transformation.

DNA transfer by natural methods		DNA transfer by artificial methods	
1	Conjugation	1	Macroinjection
2	Bacterial transformation	2	Microinjection
3	Transposition	3	Protoplast fusion method
4	Phage transduction	4	Biolistics transformation
5	Retroviral transduction	5	Liposome mediated transformation
6	Agrobacterium mediated transfer	6	Electroporation

Once the transgenic event is steady, expressed and inherited in succeeding generations, then the plant is considered a transgenic. Backcrossing is the ultimate stair in the genetic engineering course, where the transgenic crop is crossed with a variety which possesses principal agronomic characters and culled in order to attain high quality plants that express the inserted gene in a preferred fashion.

Gene Transformation

The transformation mediated by *Agrobacterium tumefaciens*, a soil plant pathogenic bacterium, has become the most used method for the exordium of peregrine genes into plant cells and their subsequent regeneration. *A. tumefaciens* naturally infects the wound sites in dicotyledonous plant to form crown gall tumors [13]. It has the unique ability to transfer a particular DNA segment (T-DNA) of the tumor-inducing (Ti) plasmid into the infected cells nucleus and stably integrated into the host genome to transcribe and cause the crown gall disease [14, 15].

The T-DNA consists of oncogenes, which encode the enzymes involved in synthesis of auxins, cytokinins and tumor formation; and opine synthesis genes. These compounds, produced by condensation of amino acids and sugars, are synthesized and oozed by the crown gall cells and used up by *A. tumefaciens* as nitrogen and carbon sources. Opine catabolism genes, involved in T-DNA transfer from the bacterium to the plant cell and bacterium to bacterium through plasmid conjugation are located outside the T-DNA [16, 17].Only the virulent strains of *A. tumefaciens* and *A. rhizogenes* interact with susceptible dicotyledonous plant cells which induce diseases kenned as crow gall and pillar roots, respectively. These strains contain an astronomically immense mega plasmid (more than 200 kb) which plays a key role in tumor induction and for this reason, it was denominated Ti plasmid and Ri in case of *A. rhizogenes*. T-DNA, a mobile segment of Ti or Ri plasmid gets transmitted to the plant cell nucleus and unified into the plant chromosome during infection. The T-DNA section is flanked by 25-bp direct repeats, which act as a cis element signal for the transfer apparatus. The Ti plasmid withal contains the genes for opine catabolism engendered by the crown gall cells, and regions for conjugative transfer and for its own integrity and stability. The T-DNA transfer process is facilitated by the compliant act of proteins encoded *vir* genes (T-DNA virulent region). The 30 kb virulence (*vir*) region is structured in six operons that are vital for the T-DNA transfer (*vir*A, *vir*B, *vir*D, and *vir*G) or for inducing transfer efficiency (*vir*C and *vir*E) [16, 17, 18].Different chromosomal-determined genetic elements have displayed their functional part in the invasion of *A. tumefaciens*to the plant cell and bacterial establishment. The loci chvA and chvB, involved in the production and emission of the b-1,2 glucan [18].The chvE is necessary in sugar enhancement for *vir* genes induction and bacterial chemotaxis [20, 21, 22].Whereas,the cell locus synthesizes cellulose fibrils [23], the pscA (exoC) locus synthesizes both cyclic glucan and acid succinoglycan [11] by their corresponding genes. The att locus is involved in the cell surface proteins [24].

***Agrobacterium tumefaciens* T-DNA Transfer process**

The process of gene transfer from *Agrobacterium tumefaciens* to plant cells implicatively insinuates several essential steps:

1) Bacterial colonization.
2) Induction of bacterial virulence system.
3) Generation of T-DNA transfer intricate.
4) TDNA transfer and
5) Integration of T-DNA into plant genome.

Future Directions

Abiotic stress is one of the major problem in rice growing area. Biotic and abiotic stresses reduce the average plant productivity upto 87% and also quality and post-harvest life. The development of abiotic stresses plants by the introduction of molecular breeding and genetic engineering seems to be a meaningful approach to hasten the breeding of improved plants.With the advent of transgenic technology, transfer of foreign genes, even from unrelated species has become possible in a very short period of time. Moreover, the farming area of transgenic crops is mounting high each year and new transgenic crop varieties are being commercially released. Although, the global acceptance of genetically improved crops are not so impressive due to lack of knowledge about the promising technology among the average population as well as the revolving myths and controversies related to safety issues worldwide. Hopefully, researchers will be able to convey answers and ensure safety issues of products to ascertain food security to the impending generations in future world.

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