Asian J. of Bio Sci. (April, 2008) Vol. 3 No. 1 : (204-208)

A Case study :

Enhancing crop productivity by biotechnology

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(Accepted : November, 2007)

Food crises in the sixties led to the development of high yielding varieties of cereals particularly, rice and wheat which resulted into green revolution and food production was increased significantly. A rapidly expanding population is a serious concern for food security especially for developing countries including that of India. To keep pace with the present population growth and consumption pattern, India's food requirement has been estimated to 225 million tons by the year 2005. Green revolution technologies, though contributing to food production, they are inadequate to meet the challenges lie ahead. Recently developed biotechnological techniques undoubtedly have major role to play in solving the food security problem, just as the green revolution had its origins in science and technology, and particularly in science of genetics. So the application of novel biotechnological tools could lead to gene revolution. With the wide spread of advanced cellular and gene technology, the new technologies will certainly help in enhancing the crop productivity.

Key words : Somaclones, Transgenic, Gene technology, Food crises, Productivity.

INTRODUCTION

The quest for agriculturist for greater productivity and improvement of existing cultivars with better quality food continues with great vigor and spirit. The success in applying conventional plant breeding principals and agricultural practices to crop improvement reaches its peak when high yielding wheat and rice varieties were cultivated in 1960's, with profound impact on agricultural production (Borlaug, 1983). The Green Revolution was successful due to the introduction of improved seeds, fertilizer, irrigation and plant protection measures combined with positive policy support, liberal public funding for agricultural research and development and dedicated work of farmers. Not withstanding all round achievements, the basic problems of food security, poverty, equity and sustainability, continues to be a cause of concern in India today (Mashelkar, 1999). The conventional methods of plant breeding and traditional agricultural practices have done tremendous job and contributed to a great deal towards the above goal. However, in view of the acuteness of the problem and renewed fears regarding the availability of the proper and enough food, these methods alone are not sufficient to meet the situation. Today, the new techniques of biotechnology in general and genetic engineering in particular may help us escaping the dangers of food scarcity for rapidly growing population of India.

Application of cellular technology :

Cellular technology or plant tissue culture is an enabling technology from which many novel tools have been developed to assist plant breeders. Tissue culture techniques are used for both, to increase the efficiency of conventional breeding methods by creating new genetic variation for crop improvement and by maintaining genetic purity of the genotype. They include micropropogation, anther culture, *in-vitro* selection, embryo rescue, somaclonal variation, somatic hybridization and transformation. Among these, somaclonal variation occupies some what unique position because it has both advantages and disadvantages of tissue culture system.

Cell or tissue culture passage has been shown to increase the frequency of mutations, thus leading to a higher production of non-uniform genotypes among regenerated plants (Murai and Kinoshita, 1986). This induced variation can broaden the existing genetic base of the crop and provide new genetic material for improvement of the genotype. Heritable genetic variation generated during the passage through tissue culture have been termed as somaclonal variation (Larkins and Scowcraft, 1981). An extensive number of reports are available in a whole range of species, indicating that somaclonal variation is widespread and therefore readily accessible to all plant breeders (Karp, 1991). Utilization of in-vitro induced novel genetic variation is one of the

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major objectives of modern crop improvement programs. Somaclones for the resistance to downey mildew, fiji and eye spot disease were identified. Application of somaclonal variation in crop improvement can produce increased yield and resistance to biotic and abiotic stresses. India's efforts have led to the development of a variety called "Pusa Jaikisan" in brown mustard evolved through somaclonal variation. Several agronomically important traits with complex genetic constitution and inheritance patterns have been modified / induced and crop species were improved by using *in-vitro* techniques (Table 1).

6. Increased benefit compared to conventional method of cultivation.

Application of Genetic Engineering and Transgenic Technology :

With the recent advances in gene technology particularly in gene cloning, *in-vitro* modification and their delivery into the other species across the fertility barriers for stable expression and inheritance has tremendous impact on the improvement of plant, animal and microbes. Gene technology enabled us to incorporate new properties into cultivated plants through inter-specific or inter-generic gene transfer, which was not possible through conventional breeding approaches. Several genes (Table 2) from microbes, insects and plants have been identified, characterized and transferred into the food crops. Transgenic is used to describe plants which have had DNA introduced into them by means other than the sexual transfer of DNA / gene from a sperm or egg cell. Transgenic provides new method of controlling insects, diseases and weed pests.

Table 1: Important traits modified through tissue culture induced somaclonal variation.

Crops	Traits Improved	Institutes
Geranium	Vigor and flower attractiveness	Purdeu University, USA
Sweet Potato	Color, Shape, Backing quality of roots	North Carolina Res. Services, USA
Sugarcane	Yield and Disease resistance	Sugar Research Institute, Fijii
Maize	Tryptophane Content	Molecular Genetics, USA
Tomato	Dry matter content, Disease resistance	DNA Pl. Tech. Of New Jersey, USA
Rice	Yield	Plant Reasech Institute, Japan
	Disease Resistance	Uni. Of Agril. Sci., Godollo, Hungary
Celery	Processing, yield and efficiency	DNA Pl. Tech. Of New Jersey, USA
Brown Mustered	Yield	ICAR, New Delhi
Lathyrus	Low toxin content	ICAR, New Delhi

(Kumar and Singh, 1999)

Recent advances in cellular technology have led to rapid multiplication and desired improvement of plants particularly horticultural and forest crops. The productivity of the vegetatively propagated plants such as sugarcane, potato, strawberry, horticultural trees and floriculture etc, reduced due to the constant accumulation of systemic diseases can be restored through micro-propagation of disease free plants in large numbers through tissue culture. The protocols for the large scale production of disease free planting material through micro-propagation have been developed for most of the vegetatively propagated plants.

Advantages of micro - propagation over existing methods of vegetative propagation or sexual reproduction are:

1. Shorter generation cycle.
2. Higher multiplication rates.
3. Year round production of propogules.
4. Elimination of disease(s).
5. Synchronous flowering and maturity of the plants generated through tissue culture.

Genetic Engineering of metabolic pathways of fruits and vegetables :

Ethylene synthesis in transgenic tomato plants using anti-sense RNA technology to a gene that has been identified as ACC Oxidase is useful in improving the keeping quality of tomato by delaying its ripening process. The enzyme converts ACC to ethylene, which is responsible for the ripening of fruit. The ethylene production was inhibited by 97% with a significant reduction in over ripening and shriveling. This ultimately increases the shelf life of fruit

Table 2 : List of Transgenic released for cultivation.

Crop	Traits modified / introduced	Year of approval / Product Name	Institution
Tomato	Delayed ripening	1994, Flavr, Savr	Calgene
	Delayed ripening	1995, Endless summer	DNA Plant Tech
	Delayed ripening	1995	Monsanto
	Thick Skin and altered Pectin content	1995	Zeneca / Pataseed
Cotton	R to bollworm & Budworm (Bt)	1995, Bollgard	Monsanto
	R to Bromoxynil	1995, BXN cotton	Calgene
	R to glyphosate	1996, Round up ready	Monsanto
	R to sulphony Urea	1996	Dupont
Soybean	R to glyphosate	1995, Round up ready	Monsanto
Potato	R to Colarado potato bectic (Bt)	1995, New Leaf	Monsanto
	Insect Resistant (Bt)		
		1996	Monsanto
Maize	R to Cornborer (Bt)	1995, Maximizer	Cibe-Geigy
	R to glufosinate	1996	Devalb
	R to glufosinate	1996, Liberty Link	Agro Evo
	Male Sterility	1996	Pl. Genetic System
	R to Cornborer (Bt)	1996, Yield gard	Monsanto
	R to Cornborer (Bt)	1996	Northrup King
Rapeseed	High Lauric acid	1996, Laurical	Calgene
Squash	R to Viruses	1995, Freedom II	Asgrow

R=Resistance

and help in long distance transportation's. Flavr Savr and Endless Summer in tomato, Freedom II in squash, High lauric acid rapeseed (canola) and Round ready soybean, are some of the examples of crops that are already being commercially grown in developing countries. Several agronomically important genes viz., seed storage protein (argenin and lysine rich), salt tolerance and herbicide resistant genes are introduced in cereals particularly rice and are under large scale field trials. Cereals, rice and wheat are main source of carbohydrate and introduction of storage protein genes into rice will increase its nutritive value and balance the poor man's diet.

Advantages of Gene technology :

1. Availability of R genes form divers source.
2. Enhanced yield.
3. Reduced farm inputs such as pesticides, herbicides etc. thus resulting in general environment protection.
4. Reduced tillage.
5. Improved quality of crops such as seed storage protein gene in rice.
6. Environmental friendly medicinal vegetable crops like, antibiotic resistance potato.

Today, genetically engineered products are available in just seven countries i.e., USA, Spain, France, Canada, Australia, Mexico and Argentina. In US who allowed transgenic products just five years ago, they already account for 50% of soybean, corn and cotton acreage. In India, cultivation and consumption of transgenic products are still not permitted by Government. India's most celebrated botanist Dr. M. S. Swaminathan, says genetic engineering could do wonders for India.

Problems associated with Transgenic :

Advances in plant gene transfer technologies led to the rapid increase in the production of transgenic plants over the last ten years. These advances have made it possible to introduce DNA (a gene) into the plants for the production of new traits such as resistance to herbicides, insects or viruses and for the study of plant genes and their controlling elements (Cramer and Radin, 1990; Grasser and Schell, 1989). However, both technical and non technical problems still exists, which limits its acceptability among large section of society.

The technical limitations for gene transfer in plants include a lack of basic knowledge about transformation and regeneration of most of the plants. These limitations are being addressed by the development of a better understanding of mechanism of agrobacterium host specificity and T-DNA transfer (Zambryski, 1988) and with the development of site specific integration vectors for DNA transfer. To overcome problems of plant regeneration, physical methods of DNA delivery by micro projectile bombardment of plant meristem and elctroporation of pollen were developed and several crops have been transformed with bacterial genes successfully.

While transgenic crops are attractive to think on a pesticide free or weed free crops, there have been cautionary notes from the biotechnologist themselves. The public opinion across world, from western countries to India is strongly in opposition for introduction of genetically modified crops for human consumption, although several large scale field trails have been conducted by various organizations. During 1986-87, 25000 field trails were conducted with transgenics of more than 60 crops in 45 countries. Sixty percent of these trials were in first 10 years and 40% in the last two years. Area under transgenics cultivation has also increased tremendously. During 1996, the transgenic crops covered an area of 2.8 million ha which increased about 4 times during 1997 and 10 times during 1998 (Table 3).

Gene silencing and expression instability:
The expression of genes in transgenic plants may not be as anticipated. The gene transferred may be expressed in an expected tissue or stage of development or at level too low to be effective. The unwnted results could be related to the genotype of the transformed plant or promoter of the gene (Schaff, 1991).

Table 3 : Area of transgenic crops planted (m ha).

S. No.	Name of the Country	1997	1998
1	United States of America	8.1	20.5
2	Argentina	1.4	4.3
3	Canada	1.3	2.8
4	Australia	0.1	0.1
5	Mexico	0.0	0.1
6	Spain	0.0	<0.1
7	France	0.0	<0.1
8	South Africa	0.0	<0.1
	Total	11.0	27.8

Resistance developed in insects to the toxic genes:
Toxins from *Bacillus thuringiensis* (Bt) provides safe and effective control of insect pests, whether in the form of topical spray or genetically engineered into crop plants. However, resistance to Bt toxins were documented in more than fifteen species of insect pests (Tabashnik *et al.* 1994) and it is possible that all species of insect have the potential to become resistant. Table 4 summarizes the laboratory experiments to select for resistance in Lepidopteran (moths), Coleoptera (beetles) and Diptera (files). The column labeled with "Resistance ratio" showed the level of resistance that was observed after the indicated number of generations of selection. One strain of *Heliothis virescens,* an important pest of cotton and tobacco showed over 10,000 fold resistant to Bt toxin gene Cry IAC.

Danger of transfer of toxic genes to wild relatives:
Taking rice as an example Rissler and Mellon, 1993 and Abbott, 1994 have raised concerns about the consequence of the spread of the transgenes from genetically engineered crop plants to its wild relatives. *Oryza sativa* (Cultivated rice) can be readily crossed with other *Oryza* species that share the AA genome. In Asia, two wild AA genome species, *O. rufipogon* and *O nivara* are spread all over the rice fields and in rain forest. O. sativa is primarily self pollinating; most ovaries on a plant are fertilized by pollen produced on the same plant. However, out crossing is more common in *O. rufipogon* and *O. nivara*. In nature extensive hybridization does take place between the *O. sativa* and *O. rufipogon* and *O. nivara* (Oka, 1991). Therefore, there are chances of transfer of transhenes from engineered rice to wild species through out crossing.

High cost of transgenic seeds:
Mostly gene technology research has been done by the private companies and requires expensive expenditure for the development of transgenic. With gene patenting and IPR cost of these seeds are not in the reach of poor farmers.

Micro-ecological changes resulting in the pest populations. Effect of toxic genes particularly Proteiases and trypsin inhibitors on human health.

The history and future of crop improvement represent a continuous effort to develop new methods

Table 4: Insect pests resistant to Bt toxins

Insect	Bt toxin gene / sub species	Generation of selection	Resistance ratio
Heliothis virescens	Cry I A (c)	30	> 10,000
Plutella xylostella	B.t. Kurstaki	17	2800
Plodia interpuctella	B.t. Kurstaki	23	140
Leptinotarsa decemlineata	Cry IIIA	35	400

which overcome the limitations of existing approaches. The application of classical genetic principles to breeding programme was and will continue to play an important role as evidenced by "Green Revolution". The success of green revolution techniques was enhanced by the development of advanced tissue culture techniques viz:, mircro- prorogation, somatic hybridization and somaclonal variation and gene technologies. The combination of conventional breeding approaches with recently developed biotechnological tools opened new avenues for crop improvement. Biotechnology based advanced technologies are expected to materialized many of our expectations in the coming millennium.

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