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Genetically Engineered Plants in Indian Agriculture

P. K. Ghosh[1]
Department of Biotechnology
Ministry of Science & Technology
C.G.O. Complex, Lodi Road, New Delhi-110 003 (India)

Summary :

Use of genetically engineered plants aims at agricultural production with higher yield potential, increased resistance to stresses and improved characteristics of fruits / crops. India has increased its agricultural production and productivities substantially over the years by the application of the conventional breeding technology, by utilising certified as well as hybrid seeds; however, the use of quality seeds has yet been low, about 9.4% of the total seed requirement. Conversion of quality seeds by recombinant DNA technology into improved cultivars hold further potential towards increasing productivities. Up to the end of 1997, eight transgenic crops involving ten traits, using 21 processes, were approved commercially, and, nearly, 25-30 million acres of land were used globally for the cultivation of transgenic crops. In India, field experiments on several transgenic crops are in progress. The current concerns from the transgenic plants are related to environmental and food safety, collectively called as bio-safety issues. The trans-boundary movement and use of genetically engineered plants in commercial agriculture are intimately linked with the global harmonization of bio-safety issues.

Introduction :

Better seed quality, several factors such as improved agronomic practices, increased understanding of plant pathology, biochemistry, Mendelian genetics, entomology, plant tissue culture techniques and development of transgenic plants with improved traits form the main factors for developing better plant varieties and increased crop production. Application of transgenic plant technology is primarily for the production of transformed plants with higher yield potentials, increased resistance to stresses and improved characteristics of the fruits / crops. Plant tissue culture is directed towards fast producing large number of clones, with specific traits, and is used, ordinarily, in situations where natural methods of propagation through the use of seeds and / or vegetative propagation are not efficient or are difficult. Tissue culture technique applied to the development of transgenic plants, helps in the emergence of better varieties in the shortest possible time.

Transfer of specific genes through well understood and properly designed vectors into target plants produce defined transgenic plants and can be called designed transgenics with specific genes transferred into them. Development of better transgenic plant varieties is through the design and invention of such plants with the target genes transferred into them.

[1] The views expressed in this paper are those of the author and they do not necessarily express the views of the organization to which he belongs.

Steps in the development of genetically engineered plants :

Development of transgenic plants has the following scientific components :

a) Identification of a gene, which would impart a useful character to the target crop plant, and cloning gene. The work, inter-alia, involves the construction and the screening of a genomic library, to fish out the gene with a probe / marker DNA.

b) Modification of the target gene for expression in the crop plants. This operation involves the selection and manipulation of a promoter, a coding sequence and the appropriate terminator sequences, with the target and marker genes.

c) Incorporation of the modified gene construct into the target plant genome, which involves transferring the modified gene into the plant.

d) Regeneration of whole plants capable of transmitting the incorporated gene to the next generation. This process takes help of the use of a selection agent, usually an antibiotic resistant gene marker. Cells not containing the resistant marker gene would get destroyed under the influence of selection pressure deliberately built in.

Factors contributing to yield and approaches of application of gene technology :

In plants, absolute yield contributions are from the following; the author from experience hypothesizes the numbers.

Factors	Contribution in %
1. Genetic make up and optimisation of gene technology	50-60%
2. Agronomic practices and agricultural technologies	25-30%
3. Biotic and abiotic stress related factors.	20-25%

There are presently three general approaches of applications of gene technology as could be stated briefly :

i. By selecting improved varieties — through genome mapping to indentify and propagate high yielding cultivars; developing somatic embryos of good varieties and micro-propagating them to generate true-to-types in large quantities; utilization of anther / pollen culture to speed up propagation of high yielding new varieties.

ii. Harnessing near-full-potential yields by developing cultivars resistant to herbicides, viruses, bacteria, fungi, pests, tolerant to salinity, drought, heat and water logging, etc.

iii. Improving existing products by directing production of economically more valuable products produced by other methods, e.g. converting rape seed/mustard to produce higher quantities of lauric acid otherwise obtained from coconut oil, converting soybean to produce more essential amino acids and reducing the content of enzymes responsible for interfering with trypsin metabolosm, transforming sunflower to produce higher oleic acid, lengthening the period of maturing of the sorghum plant to increase its feed qualities, reducing the contents of anti-nutritional substances from tomato, delaying the ripening of fruits like tomato to improve their keeping qualities, modifying cotton cultivars to improve the fiber qualities such as better moisture absorption properties etc.

Existing production and productivities :

It is necessary at this stage to look at the existing production and productivities in agriculture; the total area, production and productivities of principal crops, fiber, sugarcane, vegetables and fruits during 1990-91 and 1994-95 are as under (Table-1) (Ref. 1-3). It could be seen that there has been gradual increase in the productivities of most of the items.

Table 1 : Production (P) in million metric tons, area (A) in million hectares and productivities, (Y) in tons per hectare of principal crops, fiber, sugar cane, vegetable and fruits

Particulars	Years					
	1990-91			1994-95		
	P	A	Y	P	A	Y
Rice	74.3	42.7	1.74	81.2	42.2	1.92
Wheat	55.1	24.2	2.28	65.5	25.6	2.55
Maize	8.9	5.9	1.52	9.12	6.1	1.49
Jowar	11.7	14.4	0.81	9.20	11.7	0.78
Bajra	6.89	10.5	0.66	7.15	10.1	0.71
Ragi	2.34	2.17	1.08	2.43	1.83	1.33
Small millets	1.19	2.45	0.49	0.87	1.86	0.47
Barley	1.63	0.96	1.70	1.58	0.84	1.88
A. Total Cereals	176.4	127.8	1.38	191.1	123.1	1.55
Gram	5.36	7.52	0.71	6.21	7.26	0.86
Arhar (Tur)	2.42	3.59	0.67	2.19	3.36	0.65
Urad	1.65	3.48	0.47	1.33	3.15	0.42
Mung	1.38	3.36	0.41	1.17	3.04	0.39
Kulthi	0.59	1.46	0.40	0.42	1.07	0.39
Peas & Beans	0.55	0.58	0.95	0.63	0.74	0.86
Masur	0.80	1.19	0.64	0.80	1.14	0.70
Khesari (Lakh)	0.56	1.06	0.53	0.58	0.95	0.61
Moth	0.47	1.49	0.31	0.40	1.47	0.27
Total Pulses	14.3	24.7	0.58	14.1	23.2	0.61

Particulars	Years					
	1990-91			1994-95		
	P	A	Y	P	A	Y
Groundnut	7.10	8.67	0.82	8.26	7.92	1.04
Castor seed	0.72	0.81	0.88	0.85	0.79	1.08
Sesame	0.84	2.52	0.33	0.62	2.03	0.30
Mustard / Rape	5.23	5.78	0.90	5.88	6.23	0.94
Linseed	0.33	1.10	0.30	0.33	0.96	0.34
Safflower	0.32	0.82	0.39	0.42	0.77	0.54
Niger seed	0.19	0.61	0.31	0.20	0.59	0.33
Sunflower	0.87	1.63	0.54	1.20	1.97	0.61
Soybean	2.60	2.56	1.02	3.67	3.99	0.92
Cotton Seed	3.36	7.44	0.45	4.11	7.93	0.52
Coconut (P in million nuts) (Y in nuts / heetare)	9.73	1.48	6.60	12.20	1.67	7.31
B. Total Oil Seeds	18.61	24.15	0.77	21.42	25.26	0.85
Total Edible Oil Seeds	17.56	22.24	0.79	20.24	23.51	0.86
Total Non-Edible Oil Seeds	0.87	1.60	0.54	1.18	1.75	0.67
Cotton (Lint, 170 Kg. Bales)	1.60	7.66	0.22	2.06	7.93	0.26
Jute (180 Kg. Bales)	7.92	0.78	1.43	8.27	0.75	1.49
Mesta (180 Kg. Bales)	1.31	0.24	0.24	1.18	0.19	0.21
C. Fiber	10.83	8.68	1.89	11.51	8.87	1.96

Particulars	Years					
	1990-92			1994-95		
	P	A	Y	P	A	Y
D. Sugar Cane	241.0	3.69	65.4	271.2	3.82	71.10
Potato	18.19	1.14	16.03	17.35	1.23	14.10
Onion	4.71	0.33	14.30	5.67	0.40	14.18
Tomato	4.24	0.29	14.62	5.26	0.35	15.03
Cabbage	2.80	0.18	15.56	3.91	0.22	17.77
Cauliflower	3.00	0.20	15.00	3.78	0.23	16.43
Okra	1.89	0.22	8.59	3.99	0.42	9.40
Peas	1.30	0.18	7.22	2.31	0.22	10.50
Others	22.47	3.06	7.34	20.20	2.48	8.15
E. Total Vegetables	58.59	5.60	10.46	68.68	5.97	11.50
Banana	7.79	0.38	20.50	13.17	0.44	29.93
Mango	8.75	1.08	8.10	10.99	1.23	8.93
Citrus	2.82	0.39	7.23	3.70	0.44	8.41
Guava	1.10	0.09	12.22	1.39	0.12	11.58
Apple	1.15	0.19	6.05	1.18	0.21	5.62
Pineapple	0.77	0.06	12.83	0.80	0.05	16.00
Papaya	0.81	0.05	16.20	1.37	0.06	22.83
Grapes	0.67	0.03	22.83	0.67	0.04	16.75
Safota	0.40	0.03	13.33	0.50	0.04	12.50
Lichi	0.24	0.05	4.80	0.33	0.06	5.50
Others	4.14	0.53	7.81	13.57	2.84	4.78
F. Total Fruits	28.63	2.87	9.98	47.93	5.57	8.61

It pleases to recapitulate that India ranks well among the World's largest producers of sugar, cotton, tea, fruits and vegetables, and that there had been phenomenal increase in production and productivities of food grains, oilseeds, sugarcane, cotton and jute over the years. However, simultaneously the population has increased which in 1997 stood at about 960 million against a global

population of about 5800 million. Consequently, the Indian per capita increase in the availability of food and agricultural produce had only been marginal. Furthermore, arable land is limited and the probability of access to additional agricultural land is drastically coming down.

Of the various methods of increasing productivities, the factors related to the optimisation of genetic make up and the manipulation of transgenes into target host plants hold maximum potential. It would be necessary in this context to have an overview of the extent of use of certified varieties and hybrid seeds in the country so as to facilitate the choice of these ones where incorporation of desired transgenic traits would yield maximum economic benefits at the earliest.

Use of certified and hybrid seeds in India :

The new seed policy announced by the government, which came into effect from October, 1988, was directed towards providing the best planting materials from anywhere in the world, to the Indian farmers. So, there was concomitant mixing of local traits with improved imported varieties through the application of scientific breeding, collaboration between local companies with foreign seed companies, and multinational seed companies, some of which brought their high yielding hybrid cultivars into the country. Simultaneously, the national infrastructure on seeds through the National Seeds Corporation Ltd., the participanting states of the State Seed Corporations, the State Agricultural Universities, the State Seed Certification Agencies, the State Farms Corporation of India Ltd., and the institutes of Indian Council of Agricultural Research contributed enormously to the development of local varieties and hybrids of different crops, and particularly of cereals. The mismatch of demand and supply position got substantially improved over the period of last one decade. The seed market share in value of both the public and the private sector outfits became high and the private sector surpassed the public sector outfits in value terms. Presently, 60% in value of the quality seed market is held by the private sector although in quantitative terms the public sector holds nearly 60% share. An analysis of the market value of the certified quality and hybrid seeds made by the author for the year 1995-96 for the major crops and vegetables is presented below in Tables 2A and 2B respectively :

Table 2A : Market analysis of seeds of major crops in India (1995-96)

Major Crops	Sown Area Mha	Sowing rate in Kg/ha	Total seed req'000T	Certified/ qlty seed supply '000T (4)	Market value Rs. in million (5)
Wheat	23.98	100	2,398.0	200.0 VAR*	1.400
Rice	42.60	30	1,278.0	150.0 VAR*	1,350
Sorghum	14.50	12	174.0	20.0 HYH*	560
				20.0 VAR*	280
Pearl Millet	10.45	4	41.8	19.0 VAR*	950
Maize	5.95	20	119.0	5.0 HYH*	100
				10.0 VAR*	100

Major Crops	Sown Area Mha	Sowing rate in Kg/ha	Total seed req'000T	Certified/ qlty seed supply '000T (4)	Market value Rs. in million (5)
Chickpea	7.41	75	555.8	13.0 VAR*	
Pigeon Pea	3.62	20	71.4	5.5 VAR*	
Lenil	1.16	40	46.4	1.0 VAR*	
Black Gram	3.40	20	68.0	7.4 VAR*	
Green Gram	3.42	20	68.4	10.0 VAR*	
Peas	0.55	60	33.0	4.0 VAR*	1,636
Groundnut	0.44	150	1,245.0	75.0 VAR*	3,000
Rape/Mustard	5.72	5	28.8	8.0 VAR*	200
Soyabean	2.37	62.5	148.2	30.0 VAR*	600
Sunflower	1.64	10	16.4	6.0 VAR*	840
Castor	0.81	12.5	10.1	2.5 VAR*	22
Linseed	1.15	25	28.8	2.0 VAR*	60
Cotton	7.40	120	148.0	12.0 HYH*	4,500
				13.0 VAR*	325
Total	141.00		6,489.9	613.4	16,126

VAR*=Certified quality seeds; HYH*=High yielding hybrid seeds

Table 2B : Market analysis of seeds of major vegetables in India (1995-96)

Major Vegetables	Sown area Mha (4)	Sowing rate kg/ha	Total seed requirement in tons	Estimated Sale of hybrids (tons)	Estimated market value in Million Rs. (5)
Egg Plant	474352	125	59.2	8.25	49.5
Cabbage	239743	250	60.0	18.00	144.0
Cauliflower	446649	250	112.0	4.00	48.0

Major Vegetables	Sown area Mha (4)	Sowing rate kg/ha	Total seed requirement in tons	Estimated Sale of hybrids (tons)	Estimated market value in Million Rs. (5)
Chilly	567851	250	142.0	1.70	27.2
Cucumber	250000	375	94.0	2.81	5.60
Gourds	405218	5000	2026.0	16.30	32.60
Melons	166030	750	125.0	8.70	26.10
Okra	367986	3750	1380.0	190.00	114.0
Tomato	480713	125	60.0	2100	315.0
Total	3398542		4058.2	270.76	762.0

It is seen from Table 2A that in the case of the major crops, of the total requirement of quality seeds of nearly 6.49 million metric tons (mmt) in 1995-96, the supply of certified quality and hybrid seeds had been of the order of 0.61 mmt valued at Rs. 16.13 billion (US$ 460 million) at current costs; the usage of quality seeds was only about 9.4% of the total requirement. The vegetable seed market is smaller (Table 2B). Of the total requirement of nearly 4058 tons, only about 6.7% was made available through the sale of hybrids valued at Rs. 762 millions (US$ 21.8 million). The above picture clearly indicates that this country has enormous scope for maximizing the use of certified quality seeds and high yielding hybrids. Conversion of these seeds into improved cultivars of pest tolerant varieties or disease resistant ones or of agricultural produce of better keeping qualities by the application of recombinant DNA technology will go a long way in improving the agricultural productivities of India.

Improvement of hybrids and certified seeds by gene technology :

Globally, at least, 64 commercially important species of plants have been utilized for incorporating transgenic traits. Important among these are cotton, mustard, maize, tomato, potato, petunia, soybean, sugarbeet, sunflower, cabbage, papaya tobacco, melon, peanut, pea, pepper, Bengal gram, squash, sugarcane, rice, wheat, barley, broccoli, cucumber, egg plant, plum, apple, berry, grape, birch, poplar, eucalyptus, spruce, walnut, rubber, lupins, gerbera, gladiolus, chrysanthemum, clover, cantaloupe, flax, carnations, etc. The traits that have been targetted for genetic acquisitions by the plants could be classified broadly as 7 only (Table 3), and that the tolerance to herbicides head the list (6-9)

Table 3 : Global experiments on genetic transfer of traits in transgenic plants by recombinant DNA technology and most frequent categories of field releases (USA data only) in % upto May 1997.

i. Herbicide tolerance including male sterility (28.9%)

ii. Insect resistance (24.5%)

iii. viral disease tolerance (10.2%)

iv. Fungal disease tolerance (3.9%)

v. Product quality improvements (25.4%)

vi. Others (production of specialty metabolites/chemicals, incorporation of marker genes, stress resistance properties etc.) (7.1%)

Current global scenario on the use of transgenic plants

China was the first country to introduce in early 1990s the viral resistant tobacco and later the viral resistant tomato for commercial use (6,7). Subsequently, in May 1994, Calgene Inc., USA introduced its Flavr Savr ™ delayed ripening tomato for commercial use (6,7). As compiled by the author, by the end of December, 1997 eight transgenic crops involving 10 traits, using 21 processes were approved for commercial use in USA (Table 4) (Ref. 7,9).

Table 4 : Transgenic Crops Approved in USA for Commercial use (TM= Trade name)

Products	Genetically Altered Traits, the introduced genes, alongwith origin of genes	Company	Year of approval & product name, if any
Tomato	Delayed ripening, polygalacturonas production in tomato, rearranged and reversed to minimize its expression by anti-sense technology	Calgene Inc.	1994, Flavr Savr™
	Delayed ripening, fragment of the aminocyclopropane carboxylic acid synthase gene from tomato	DNA Plant Tech.	1995, Endless Summer™
	Delayed ripening, aminocyclopropane carboxylic acid deaminase gene from Pseudomonas chloraphis 6G5 strain	Monsanto Co.	1995
	Thicker skin & altered pectin content, fragment of polygalacturonase gene from tomato	Zeneca/Petoseed	1995

Cotton	Bt gene incorporated plants (boll worm & bud worm resistant), Cry1A(c) gene from Bt kurstaki	Monsanto Co.	1995, Bollgard™
	Resistant to bromoxynil, by nitrilase gene from Klebsiella ozaenae	Calgene Inc.	1995, BXN Cotton
	Resistant to glyphosate, enolpyruvylshikimate-3-phosphate synthase gene from Agrobacterium sp. strain CP4	Monsanto Co.	1996, Round up ready™
	Resistant to sulphomoyl urea, acetolactate synthase gene from tobacco (Nicotiana tabacuum cv. Xanthi)	Dupont	1996
Soybean	Resistant to glyphosate, enolpyruvylshikimate-3-phosphate synthase gene from Agrobacterium sp. CP4	Monsanto Co.	1995, Round up ready™
Potato	Bt gene (Colorado potato beetle resistant), CryIII(A) gene from Bt. tenebrionis	Monsanto Co.	1995, New Leaf™
	Insect resistant (Bt gene incorporated), modified CryIII(A) gene from Bt.	Monsanto Co.	1996
Maize	Bt gene incorporated (resistant to corn borer), Cry1A(b) gene from Bt. Kurstaki	Ciba-Geigy Corp.	1995, Maximizer™
	Glufosinate Resistant, phosphinothricin acetyl transferase gene from Streptomyces hygroscopiens	DeKalb Genetics Crop.	1996

	Resistant to glufosinate,/ phosphinothricin acetyl transferase gene from Streptomyces viridochromo	AgrEvo Inc.	1996, Liberty Link™
	Male sterility, barnase gene from Bacillus amyloliquefaciens	Plant Genetic Sys.	1996
	Bt gene incorporated (resistant to corn borer), Cry1A(b) gene from Bt. Kurstaki	Monsanto Co.	1996, Yield Gard™
	Bt gene incorporated (resistant to corn borer), Cry1A(b) gene from Bt. Kurstaki	Northrup King	1996
Rape seed/ Canola/must ard	Altered oil composition (high lauric acid content), 12:0 acyl carrier protein thioesterase gene from Umbellularia californica	Calgene Inc.	1995, Laurical™
Squash	Resistant to viruses, coat protein genes of watermelon mosaic virus 2 and Zucchini yellow mosaic virus	Asgrow Seed Co.	1995, Freedom II™
Papaya	Resistant to viruses, coat protein gene of p type of PRSV HA-5-1 from Hawaii	Cornell Univ., USA	1997

The developments in many other countries have also been phenomenal. Discussion with people from different parts of the world dealing with transgenic crops research revealed that there had been increase in the use of land for transgenic crops, which was negligible in 1995 but was about 4 to 7 million acres in 1996 and about 25 to 30 million acres in 1997, with approximate break up as in Table 5.

Table 5 : Country wise land use for transgenic crops in 1995, 1996 and 1997, million acres

Country	1995	1996	1997	Major transgenic crops planted
USA	0.1	3-4	18-20	cotton, corn, soybean, tomato
China	0.1	0.21-1	2-4	tobacco, tomato
Canada		0.8-1	3-4	mustard, corn, potato
Australia		0.08-0.1	0.08-0.1	cotton
Argentina		0.2-0.3	2-2.2	soybean
Mexico	-	0.1-0.15	0.1-0.15	cotton, tomato
Other	-	negligible	negligible	various crops
Total	0.2	4.3-6.55	25.18-30.45	

Countries are permitting the commercial use of transgenic plants after they are "satisfied" about the bio-safety aspects from their use. The "approved plants" must have also provided additional economic advantage in experimental trials by manifesting the propeties imparted upon then through recombinant DNA technology eg. herbicide tolerance, increased insect resistance, specific viral disease resistance etc. It is too early however to be assured with certainty that in every case of large scale use, the economic advantages are real and sustainable, till the world community uses these plants extensively over long periods. In the meantime, concerns had been raised on the utility of Bt cotton in USA (10) after observing the boll worm damage in the fields of transgenic Bt cotton in Texas in July, 1996. Subsequently, it is understood that Monsanto had undertaken extensive studies to ascertain if the boll worm damage was due to the development of resistance of H. zea and H. virescens to the Bt protein. Personal discussion with the scientists revealed the according to them the preliminary data indicated that there was no change in the sensitivity of the Bt proeins to susceptibility to these pests. The Bt proteins expression level in the cotton plants was also consistent with the earlier data. However, that change in weather conditions to hot and humid state for a considerable period has a dominating role to play in deciding the extent of protection of the plants from attack from boll worms was substantially established from the happenings of 1996 in the transgenic cotton fields of several parts of USA. The current tempo is to maximize the use of transgenic plants at least in certain industrialized countries. The use of such plants with these imparted agronomic traits is therefore anticipated to increase globally in future, specially due to the selling pressure comming from such countries.

Indian scenario on transgenic plants :

The first transgenic plant experiment in the field was started in 1995 when Brassica juncea plants containing Barnase, Barstar and Bar genes were field planted at Gurgaon (Haryana) to assess the extent of pollen escape. Subsequently, several experiments have been started in the field in different locations using transgenic mustard, cotton, cabbage, cauliflower, tolacco and tomato. Several Indian institutes and organizations have claimed to have developed transgenic plants which are ready for green house / screen house / poly-house evaluation and some are ready for field evaluation as well. The following Tabel-6 gives a gist of the major Indian development up to the present time (March, 1998) in transgenic plants.

Table 6 : Indian developments in transgenic research and applications

Institute	Plants / crops used for transformation	Transgenes inserted	Aim of the project and progress made
Central Tobacco Research Instt., Rajahmundri	Tobacco	Bt toxin genes : Cry1A(b) and Cry1C	To generate plants resistant to H.armigera and S.litura. Ready to undertake field evaluation.
Bose Institute, Calcutta	Rice	Bt toxin genes	To generate plants resistant to lepidoptern pests. Ready to undertake Green House testing.
Tamilnadu Agricultural Univ., Coimbatore	Rice	Reporter genes like hph or gus A	To study extent of transformation.
South Campus Delhi University, Delhi	Mustard / rape seed	Bar, Barnase, Barstar	Plant transformations completed and ready for green house experiments
South Campus Delhi University, Delhi	Rice	Selectable marker genes eg. hygromycin resistance and gus gene	Gene regulation studies. Transformations completed.
National Botanical Research Institute, Lucknow	Cotton	Bt toxin gene	To generate plants resistant to lepidoptern pests. Lab. transformations in progress.

Indian Agricultural Research Institute sub station at Shillong	Rice	Bt toxin gene	To impart lepidopteran resistance, transformations in progress.
Central Potato Research Institute, Simla	Potato	Bt toxin gene	To geneate plants resistant to lepidopteran pests. Ready to undertake Screen House trials.
M/s Proagro PGS (India) Ltd., New Delhi	Brassica / Mustard	Barstar, Barnase, Bar	To develop better hybrid cultivars suitable for local conditions; field trials in progress at multi-locations
M/s Proagro PGS (India) Ltd, New Delhi	Tomato	Cry1A (b)	To develop plants resistant to lepidoptern pests; glass house experiments in progress
M/s Proagro PGS (India) Ltd., New Delhi	Brinjal	Cry1A (b)	To develop plants resistant to lepidoptern pests; glass house experiments in progress
M/s Proagro PGS (India) Ltd., New Delhi	Cauliflower	Barnase, Barstar and Bar	To develop hybrid cultivars for local use; glass hous experiments in progress
M/s Proagro PGS (India) Ltd., New Delhi	Cauliflower	Cry1H/Cry 9C	To develop resistance to pests; glass house experiments in progress
M/s Proagro PGS (India) Ltd., New Delhi	Cabbage	Cry1 H/Cry 9C	To develop resistance to pests; glass house experiments in progress
M/s Mahyco, Mumbai	Cotton	Cry1A(c)	To develop resistance against lepidoptern pests; Multi-centric field trials in progress

M/s Rallis India Ltd., Bangalore	Chilli	Snowdrop (Galanthus nivalis) lectin gene	Resistance against lepidopteran, coleopteran & homopteran pests; transformation experiments in progress.
M/s Rallis India Ltd., Bangalore	Bell peper	Snowdrop (Galanthus nivalis) lectin gene	Resistance against lepidopteran, coleopteran & homoptern pests; transformation experiments in progress
M/s Rallis Inida Ltd., Bangalore	Tomato	Snowdrop (Galanthus nivalis) lectin gene	Resistance against lepidopteran, coleopteran & homopteran pests; transformation experiments in progress.
Indian Agricultural Research Institute, New Delhi	Brinjal Tomato Cauliflower	Bt toxin gene Mustard / rape	To impart lepidopteran pest resistance, transformation completed and ready for green house trials

Indian efforts in research on transgenic plants :

Government of India from the Department of Biotechnology (DBT) has been the prime mover for the propagation of research in transgenic plants and for the development of transgenic cultivars in the country. During 1989-97, nearly Rs. 270 million or nearly 4% of its total budget, was spent by DBT alone on plant molecular biology research, where the projects primarily focussed on developing transgenic plants of higher economic value. There had also been marginal support from the Indian Council of Agriculture Research, Department of Science & Technology, Council of Scientific and Industrial Research and certain other agencies. The combined expenditure of all these agencies would not exceed Rs. 70 million. The private sector units have started allocating some funds during the last three years and their investment could be estimated to be of the order of Rs. 100 million. Thus, the total money allocated for research in transgenic plants in the country could be estimated to be about Rs. 440 million up to the present time. It is anticipated however that substantial investment would be made from the private sector soon, in coming years.

Current concerns from transgenic plants :

The behavior of a transgenic plant in the open environment cannot be precisely predicted. Genes coding for specific properties and characteristics, unrelated to the natural plant, are made to become an integral part of the transgenic plant genome by applying recombinant DNA technology, also

known as gene technology. In the process, the new gene stretch is to be adopted through generations in a different genetic environment. This situation is sepected to be manifested in newer behavioral characteristics which can not be predicted over a short span of time.

Transgenes expressed in specific genotypes may show variations in expression when back-crossed with near relatives. The level of expression could therefore be different in different near relatives although the genes may be the same. Environment (heat, humidity and light) has also a profound role to pay to impart control on the levels of expression. Results of assessment of transgenic plants containing specific genes in one environment may not be valid therefore in other environment. Proximate analysis of the transgenic and the non-transgenic cultivars would enable to assess the consititutional changes if any, in the two plants grown in different agro-climatic locations.

A transgIne in a host in an environment is the subject matter of environmental biosafety concern. Change is any one of these three factors calls for a fresh environmental safety evaluation.

Pollens are known to travel up to certain distances in the open environment by wind as well as by the pollinator insects. Such distances of travel of pollens vary from species to species. Maintenance of proper isolation distances would ensure arrest of transgenic pollens into the wild where they are implicated with doubtful safety issues.

Some gene expressions in natural conditions are safe. However, expression of such genes in unnatural hosts may make them different, and, may thus raise newer questions of safety. The insecticidal crystal proteins expressed by various genes obtained from different species of Bacillus thuringiensis (Bt) are usually crystaline insoluble inclusion bodies found over the spores of the bacteria. These proteins are classified as Cry and Cyt toxins. The intact proteins have been found to be generally safe to mammals in feeding studies when used as "insoluble proteins". However, "in soluble forms" several of the "Bt proteins", specially the Cyt toxins, have been found to reduce the activity of memberane bound proteins of the human erythrocytes in *in-vivo* studies, and, further, they have been implicated with decreased resistance to hypotonic lysis of the cells and to cause increased membrane lipid peroxidation (11-14). The "Bt proteins" expressed through the Bt genes into plants are usually the solubilised proteins present in the cytoplasm of plants/plant parts as the genes are modified to direct the expression of only the required parts of the protein which are responsible for insect toxicity. Such modified proteins may have safety implications when admixed in human/mammalian food chain. *In-vivo* experiments in animals are therefore necessary. Case-by-case analysis is accordingly required to be carried out before specific Bt genes could be consisdered as safe. Currently, according to the author, only Cry`A, Cry1A(a), Cry1A(b), Cry1A(c), Cry1B, Cry`H/Cry9C, CryIIA, CryIIIA, CryV and CryVIa genes have been extensively used for transforming agricultural plants; in some of these publications, the full authenticity of these genes have not been disclosed. It is not clear whether these are the exact equivalent of the natural genes or some modifications have been made to use near natural Cry genes. Data on the residence time of all the gene products in gastric juice in lab conditions *in vitro* (simulating *in vivo* gastric conditions) are also not available. These information are intimately related with the safety characterization of the proteins being expressed by these genes.

Viral disease resistant plants may provide opportunites to evolve newer virulent strains of plant viruses by recombination between the viral genes contained in the modified transgenic plants and other infecting viruses against which the target plants are not protected.

While constructing transgenic plants, usually the transgenic construct has marker genes and enhaner genes. These genes code for specific proteins which are impicated with, say, antibiotic re-

sistance, resistance to certain stress conditions otherwise non-encountered by the natural genotypes. The safety implications of these genes and products therefrom are also to be ascertained from different angles.

The transgenic plants containing different "unnatural" proteins produced by the transgenic expression of human introduced genes in the target plants would produce flowers containing transgenic proteins in various parts of the flowers. Presence of such proteins is expected to alter the behavioral pattern ofthe pollinating insects.

The main concerns are therefore the right assessment of the magnitude ofthe consequences from the use of genetically modified plantsto the habitat including human and animals, the flora and fauna, and the environment. The concerns are that chemical herbicide resistant plants may confer new properties to the near relative wild plants to make them too resistant to herbicides by transferring the resistant pollents to them and thus may transform them into plants, resistant to the currently used chemical herbicides. This situation would not be controllable by the known chemical herbicides.Therefore, agricultural production would become more expensive. Viral disease resistant plants may provide opportunities to produce newer virulent plant viruses, by recombination between the viral genes contained in the modified transgenic plants and other infecting viruses against which the target plants are not protected. Insect resistant plants developed by incorporating specific *Bacillus thuringiensis* (Bt) genes, coding for proteins toxic to pests, into plants may create situations of fast loosing the bio-pesticide value of the gene products (and therefore the genes), as resistant insects are expected to be developed faster than is surmised, by excessively deploying such transgenic plants in the open environment(8). Such possibilities are expected to be witnessed earlier in these areas where over large stretch, only monoculture crops are raised. Besides, there are also concerns that the use of some of these plants and their products in the human food chain could prove allergic to some people (15)

Assessment of biosafety of transgenic plants

As mentioned earlier, the use of recombinant DNA technology in agriculture is expected to improve the yields of the agricultural products by speeding up the growth cycle of plants, by changing the physical look and structure of the agricultural products by bringing in uniformity, thereby imparting saving in labor and land use, by incorporating tolerance to adverse conditions of soil and weather, by improving resistance of plants to pests, pathogens, herbicides etc., and by creating new seeds thereby producing existing products through alternative agriculture e.g. coconut type oil in brassica etc,. The dichotomy between the acceptance of the new technology for commercual gains and the questions of safety are intimately interrelated. The decisions for the application of transgenic plants in commercial agriculture would certainly require the satisfactory answers to the concerns of safety among all cross sections of human being. Decreased familiarity about the risks associated with their use aggravates the situation further. The concerns assume great significance particularly for applications in agriculture, as the products would be parts of human and animal food chain. It is for these primary reasons that the questions of safety from the application of transgenic plants are being passionately posed by different countries in different forums.

In order to be assured about all aspects of safety from the use of new technologies, it is surmised that certain steps taken sequentially, and answers found on sound scientific assessment would resolve all the issues concerning safety (7,16). It is mentioned in this connection that steps should be taken graduallyand steadily without showing an ioata of hurry and rush to reinstate the process of confidence building among all sectors of human community. In all initial assessments in the open environment, contained experiments must first be

carried out. The purpose of containment is to ensure fool proof mentods of maintaing control over the spread of the genetically modified organisms (GMOs) in the open environment. Further, it is to reduce the exposure of the GMOs to the personnel handling them, particularry in cases where the GMOs are associated with any human allergens.

It would therefore be prudent to generate information on the characteristics of the donor organism providing the target gene or the nucleic acid stretches used in the genetic manipulation, the characteristics of the vesctors used, the nature of the transgenic inserts and the properties of the transgenic plants. The information on the donor organism for the nucleic acid stretches should include the identification, characterization of geographical origin, distribution and survival mechanism of the organism, its pathogenicity and toxicity characteristics to plants and amnimals if any, allergenicity characteristics to human or animals, and the method of transfer of its genetic materials to other organism. These information would ensure valuable insight into the risks that may be investigated before the genes and products therefrom, could be considered as safe. The information on the vectors used should include their origin, indentity and habitat, the nucleic acid sequence, frequency of mobilization, and specificity and ability to get established in other hosts. The information about the presence and the details of marker genes in the vector is also equally important for the safety assessment point of view. On the transgenic inserts, the details about the specific functions played by the inserted nucleic acid including the marker and enhencer genes if any, should also be available. The information must include the toxicity, allergenicity and anti-nutritional data of the gene products to human and animals. On the characteristics of the transgenic plants, the information on the methods of detection of such plants in the environment and the methods of detection and characteristics of the escaped transgene traits into the open environment would provide post monitoring capabilities. During the initial field trials, data on the toxicity of gene products on the hosts plants and the near relatives (in case of pollen excape and pollen rehabilitation therefrom) as well as the toxicity and allergenicity of gene products to human and animals need to be generated.

Pollen escape, pollen transfer to the wild near felatives and the consequences to the environment needs to be determined by well designed experiments. Concurrently the pathogenicity, toxicity, allergenicity and anti-nutritional properties of the plants and fruits to human and animals are also required to be looked at and data seeking answers on them are needed to be generated.

Discussion and conclusions :

It is assessed that it world be possible to precisely document on the characteristics of the donor organism providing the target nucleic acids, the vectors used, the characteristics of the transgenic inserts, and finally on most of the characteristics of the transgenic plants. On information on the flow of transgenic pollens and their establishment into the wild in the near relatives, there already exists past information on the extent of gene escape for many crops. Consequently, safe isolation distances have been worked out (17) in the context of generating breeders seeds which information could form a basis for designing newer experiments for assessing the extent of gene escape. Data commensurate with local conditions could be generated to take steps to ensure safety from the use of transgenics in the open environment under deregulated conditions. In instances where pollen escape for the transgenes would thereaten the envoronment, their use may be disapproved. For others, there should be no hesitation to use them as the deregulated seeds or plants or plant propagules.

Transgenic plants which enter into the human food chain directly or indirctly raise concerns of food safety. Substances used as food should be

proven to be totally safe. Toxicity information not only includes chronic toxicity studies but in doubtful situation may also require to generate information on genotoxicity, teratogenicity, neuro-toxicity and immuno-toxicity status. Substances with no significant past consumption as food raises concerns of safety. Therefore toxicity and allergenicity data would have to be generated using scientifically acceptable protocols. Generation of toxicity data has several components and a number of animal models are available. Thus, for acute oral toxicity studies in rats the guidelines (18) of the Organisation of Economic Cooperation and Development (OECD) No. 401. could be used. The LD50 values and their range could be calculated by the well established procedure (19) and similarly the toxicity rating (20). The sub acute toxicity in rats could be studies as per the OECD guidelines No. 404. Acute and sub acute toxicity studies may be necessary to be studied in large ruminanants abundantly available in the local ecosystem, and animals like cows, goats, sheep, pigs, dogs etc. could be selected, and data recorded using OECD guidlines No. 408 as the basis. For the avian species the acute toxicity evaluation, the median lethal dose and the likely hazards could be studied in pigeons or parrots or other representative birds in the local ecosystem; the toxicity rating could be done by well established procedures (20) and similarly for median lethal dose calculations (19). Skin sensitivity in guinepigs could be studied as per the OECD guidelines No. 404. Irritation of mucous membrane in female rabbit vagina could be studied as per OECD guideline No. 405. For studying allergenicity there exists several models such as IgE responses in mouse model (21), anaphylaxis in rat (22) and guinea pig (23-24) models and dog model (25) for asthma.

Animal models are generally acceptable by the scientific community but are not often fool proof. These models often fail to predict if unknown proteins would actually be allergenic to human. Our understanding of what makes proteins allergenic is yet inadequate. Having regard to other economic benefits for the use of transgenic plants and fruits, we may start using transgenic food after generating some minimum base-line information on animals and become vigilant about them during their subsequent use in human food chain. One thing is therefore clear that during intial introduction there would be no escape from leveling the genetically engineered food, although segregation in practical working may be extremely difficult. On the other hand, certification of safety, contents and quality by the marketer of genetically modified products would be a strong assurance to the users for facilitating acceptance. It is not clear however, to what extent the marketers; this factor would assume the responsibility of liabilities would indeed delay the acceptance of transgenics in many parts of the world. Once introduced and accepted, the labeling requirements may get withdrawn gradually thereafter.

Concerns about new food to cause allergy should be addressed by starting from examining the similarity of the amino acid sequence of the transgenic protein with the known sequences of allergens. The amino acid sequence of allergens available from the databases could become handy for the purpose. One of the source of the data is Gene Bank/EMBL/Genpept ver 86.0, SWISSPROT ver 30, PIR ver 41 and has been compiled recently (26). Usually the minimum length of peptides of a new protein showing similarity of amino acid sequence in 10 to 12 numbers at a stretch with an allergen could be a protein of suspect for mainfesting allergenicity. Such minimum stretches of congruence of amino acids or slightly longer ones could form epitopes for binding on T-or B-cells. Binding of proteins to cells is considered to be the first step for a protein to mainfest allergenicity. Sequence matching for a new protein with known allergens could therefore be a criteria for its initial evaluation as an allergen. In case no matching is observed, the next steps of animal experiments could be resorted to. The chances of predicting the allergenicity potential of the new proteins in the animal experiments

are less unless the number of animals tested is large, as very small percentage of the animals in a population is anticipated to be the responders. In human, allergy occurs in less than 2% of population for the intake of specific allergenic food proteins (which are known to cause allergy in some people, like intaking egg, milk, soybean proteins etc.) Negative evidence from these systematic steps would increase the chance of acceptability of the transgenic platns or fruits of products therefrom for use by the humankind.

As regards anti-nutritional properties of transgenic plants, the minimum that could be done is to have a detailed proximate analysis of the transgenic as well as the non-transgenic varieties. The information should include the moisture content, the dry matter, ash, the percentage of proteins, carbohydrates, fats & oils, minerals, specific anti-nutritional factors etc. For the proteins, as far as possible the amino acid analysis should be available. For the carbohydrates, the consituent sugars could be assessed and for the oils & fats the fatty acid composition could be determined. Comparison of the constituents for both the transgenic and non-transgenic varieties would enable to conclude on the near equivalence status of the transformed plants. Any change in the anti-nutritional factors would raise concerns of safety.

It could be observed from the text that the work on transgenic plants has started in some Indian institutes, and the work is largely to transform plants containing Bt toxic genes. The three private companies involved namely M/s Pro-Agro PGS India Ltd., New Delhi, M/s MAHYCO, Mumbai and M/s Rallis India Ltd., Bangalore are working on transgenic experiments utilizing the advanced knowledge made available tothem from their respective foreign collaborators. Substantial progress in field evaluation with different transgenic plants has been made both by Pro-Agro and MAHYCO, both experimenting with plants transformed by their collaborators outside the country. It is anticipated that some transgenic plants through private sector efforts and a few through public sector efforts are expected to reach the deregulated status within a couple of years after the information on safety are generated. Taking into consideration, the current state of preparedness in the generation of experimental field data and food safety information, it is anticipated that transgenic mustard containing Barstar, Barnas and Bar genes may reach the commercial market by 2000 AD. Transgenic cotton containing Bt toxic gene as also transgenic tobacco containing Bt genes may also be commercially available in India by that time. By 2001 AD, a number of products such as transgenic tomato, egg plant cabbage and, cauliflower containing different Bt genes may also be available commercially. Other products like transgenic rice, wheat, sugarcane etc. may acquire the capacity to appear only after 2001 AD, as the developments are yet inadequate.

The Department of Biotechnology of Ministry of Science & Technology, Government of India, has spent nearly Rs. 6750 million during the last ten years, from 1986 up to March 1997, in various aspects of promotion of biotechnology including development of trained manpower, creation of sophisticated infrastructure for research, support for R&D, demonstration, technology development, patenting and infrastructure for research following bio-safety guidelines. For research in transgenic plants during 1989-97 March, a sum of nearly Rs 270 million was spent by the Department of Biotechnology alone. These spread out and concerted efforts would enable the country to develop and absorb transgenic plant biotechnologies faster than many other developing countries. The results currently showing through research publications would get substanitially magnified in applcations with more industries getting interested in this area in future, as economically these products are expected to contribute positively in bringing in more prosperity to the country in agriculture and allied areas.

The road to success towards the use of transgenics in commercial agricutlture is not going to be smooth if risks are assessed by scientists

alone. There is also much discussion at international level on establishing the rights and obligations of the various parties who have interest in some form in these issues (27). The WTO's agreement on Trade Related Intellectual Property Rights (TRIPS) are primarily concerned with the rights of innovators and are concentrating on how intellectual property rights could be upheld (28). The Convention on the International Union for the Protection of New Varieties of Plants (UPOV) is focusing on the plant breeders' rights and have some consideration for the researchers as well as the farmers (29). The Convention on Biological Diversity (CBD) on the other hand is concerned with Sovereign Rights and Community Rights on genetic bio-diversity and suitable compensation to the providers by the users in the event of commercial gains from the use of genetic bio-diversity (30). All these International Conventions in certain clauses differ from one another and unless there is uniformity and mutual harmony in all these conventions it will be difficult to take care of the interests of all classes of people through out the world. Added to these is the need to harmonize national laws with these international conventions so as to work out a minimum common acceptability criteria to enable us to utilize the benefits of new technology through the implementation of acceptable global bio-safety protocol usable by all countries concurrently, especially for the transboundary movements of such products.

Use of transgenics in commercial agriculture is intimately linked with global harmonization of bio-safety issues. Currently the bio-safety issues are being looked at purely from scientific angle and all questions being asked are seeking answers only to scientific queries. There are however, many issues which would have ethical, political, cultural and social angles. The present situation offers opportunities for continuous negotiations and learning. It is surmised that the various societal forces, the commercial organizations, the scientific community and the people of different walks of life shall have profound say on the eventual acceptance of bio-safety protocols and transgenic plants. The growing preference of consumers for naturally grown food stuffs in the developed countries are indications of concerns for the use of transgenic plants in human food. The new biotech products may soon enter into several corners of the agricultural lands faster than is currently surmised; India would be no exception to this. The mankind would therefore be on the threshold of anxiety awaiting to face the new challenges that may emerge from the use of gene technology in agriculture. In the meantime, for a while, during the next one decade different countries, standing at divergent agroclimatic and genetic bio-diversity situations, are anticipated to take a case-by-case approach on a cautions mode to authorize the spread of this technology in their countries.

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