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Towards Engineering Transgenics Tolerant To Biotic and Abiotic Stresses in Crop Plants – A Review

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ABSTRACT: Abiotic and biotic stresses namely drought, submergence, salinity, alkalinity, metal toxicity, and insect pests including nematodes, disease causing pathogens including bacteria, viruses, fungi etc., are very difficult to tackle by adopting simple conventional strategies, which are costly, recurrent and eco-offensive. Traditional measures are niche-specific and exhibit tremendous vulnerability to the climactic conditions due to varied intensity of edaphic factors. Keeping this in purview, the present article makes a serious attempt to accumulate the available information and finally come out with a sound strategy by deploying cutting edge science and modern biotechnological approaches paying special attention towards development of large scale cultivation of transgenic crops, which is deemed to be conclusive by halting menaces caused by biotic and abiotic stresses to enhance productivity especially at the interface of global climatic changes, which are amplifying alarmingly, which would lead to acute food and nutritional insecurity which would fail to sustain an livelihood.

KEYWORDS: Crop plants, abiotic stress, biotic stress, tolerance, transgenics.

I. INTRODUCTION

Agriculture is an embodiment of human civilization and global unity. It provides the lifeline for sustenance and perpetuation of all living beings. The genesis of agriculture was perhaps implanted unconsciously at the dawn of civilization, when the pre-historic hunter-gatherer human beings were heavily engaged in the race for their survival. It is assumed that during the last 2000 years, agriculture has undergone radical changes in which, both natural and man-made efforts are intimately associated. The resultant being that we have more than 100 edible major crops in different continents, which provide the lifeline fuel for our survival perpetuation and prosperity. It is also very interesting to note that the pre-historic man, who were heavily dependent on the flora and fauna as a source of their livelihood, conceptualized the idea of selecting better plants and in course of time thousands of germplasm got exchanged among the continents through the then human beings, which subsequently have been improved by applying different tools and techniques to support the demand for food, cloth and shelter. Modern agriculture is not very old, however, spectacular achievements have been made in collection, characterization, utilization and value addition of germplasm for higher productivity and premium quality to support life. Man-made selections also gave birth of a number of elite entries in diverse crops, which are being used over centuries across the world. Nonetheless, owing to uncontrolled population growth our demand is widening day by day to meet the fallout between the production and requirement, which can be readdressed with the help of conventional breeding and management practices, thereby revolutionizing agriculture. Evolution of course has taken its toll by wiping many of the species, while some having been labeled threatened and endangered, which is shrinking the bio-diversity and we are losing many immensely important species day by day. On the other hand, there is hardly any scope of horizontal expansion of the land to augment agriculture since land gets diverted into non-productive plots especially for industrialization and urbanization. It is to be mentioned that at the

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interface of climate change caused mainly due to natural and anthropogenic reasons some of which include deforestation, emission of green house gases, etc., the smooth survival of our planet is at stake. As a consequence of which, the flora and fauna are also getting affected immensely. There is tremendous change in the intensity and abundance of abiotic and biotic stresses, which is making agriculture non-remunerative and less productive even though more energy is being spent by applying chemical fertilizers, pesticides and other additives. The annual precipitation pattern and amount is regularly changing across the years, which has made agriculture more difficult. Newer insects and disease pests, which were once upon considered minor have assumed the shape of predominant and are making heavy dent in the annual productivity, which is not only eco-offensive but damaging our life through altered food and nutritional inputs. At the interface of the availability of modern tools and techniques and availability of vast treasure trove of knowledge in respect of agriculture, merging both conventional and cutting edge frontier science synergistically and by timely deployment of the most sophisticated tools and techniques, including molecular biology, nanotechnology, bio-remediation etc. can bring an effective cessation of such biotic and abiotic stresses caused via anthropogenic and naturogenic modes to enable facilitation of enhanced productivity and nutritional security to mankind [1].

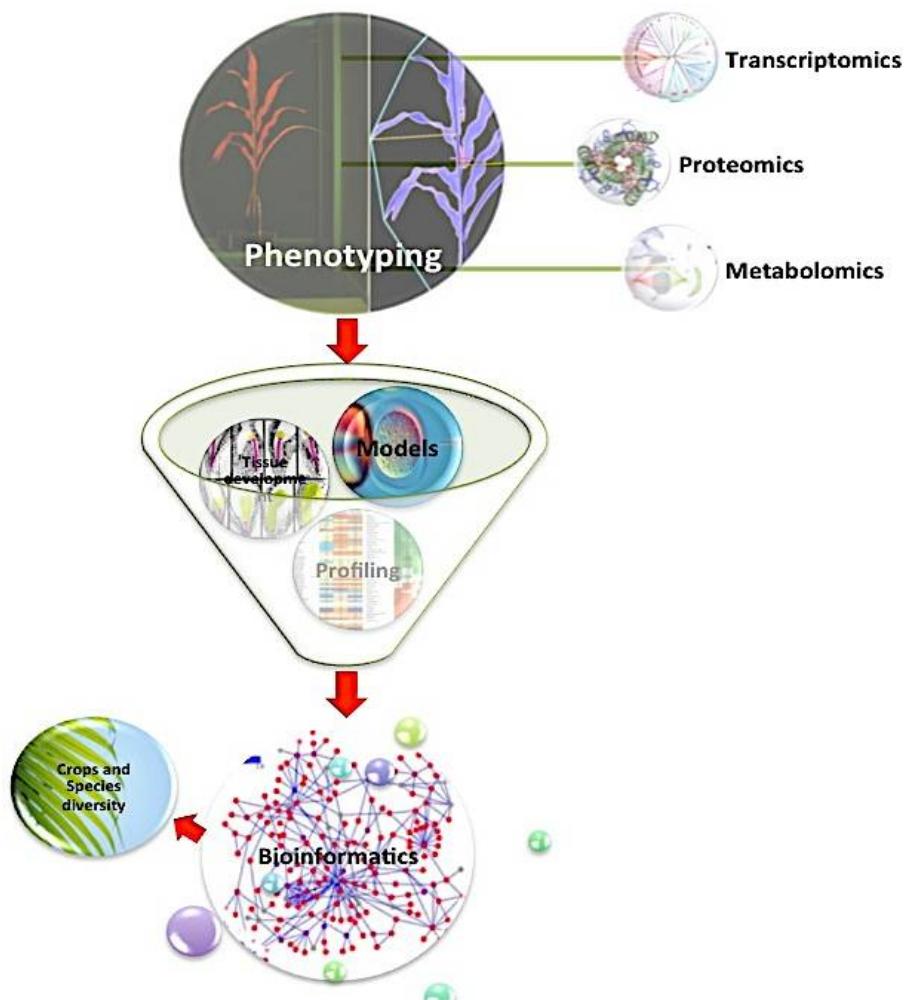


Fig.1 The flow of information and understanding from molecular level in crops and various frontier technologies used step-by-step. Source:Bohnert et al. 2006 [30].

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II. ABIOTIC AND BIOTIC STRESSES

Life took its birth millions of years ago on this planet. Since inception it has undergone tremendous metamorphosis both at structural and functional levels owing to a variety of reasons, be it naturogenic or anthropogenic that ultimately led to the evolutionary process and immense complexity arose in course of the time scale. The changes, which have occurred probably were obviously indispensable, to provide added advantage for survival and perpetuation of the concerned species at a given time. Changes occurred in every aspects of life, which could be conventionally categorized as micro and macro level changes. This is generally designated as evolution, which is continuously taking place. To the contrary of continuous changes, incidence of sudden discrete and discontinuous changes have also accomplished mutation and had been a great source of variation though the quantum of such changes gleaned to be 10^7 in *natura*, as evident from fossil data. Such changes are found to be omnipresent and regardless taxa or species, plant or animal, bacteria or virus. The causes and reasons in accomplishing changes in life forms are immense. It was observed at different geological time scale that such changes had different gradients and intensities. In recent times along with naturogenic, a large number of factors of anthropogenic origin became imperative, which plays a key role in driving diverse phenomenon to induce changes in the realms of abiotic and biotic stresses.

II.1. ABIOTIC STRESS

In agriculture, among the abiotic stresses, drought, salinity, alkalinity, submergence and mineral toxicity/ deficiencies are serious impediments in lowering both productivity and production. Abiotic stresses like salinity, low temperature and drought in particular- are responsible for most of the reduction that differentiates yield potential from harvestable yield [2]. Salt stress leads to changes in development, growth and productivity and under severe stress survival is threatened. Salinity is a significant complication affecting about a third of the irrigated land [3] and limiting the yield potential of modern cultivars. It has been estimated that salts affect nearly 950 million ha of land globally. Soil salinity constitutes a major abiotic stress in agriculture worldwide and possesses profound detrimental effects on productivity. Conventional selection and breeding techniques have been used to improvise salinity tolerance in crop plants [10] but found to be partially successful in transferring salt tolerance to the target species [11] primarily due to the multigenic origin of the adaptive responses. In India about 8.6 million ha [4] of land area is found to be highly prone to salinity. Nearly 20% of the world's cultivated area and nearly 50% of the world's irrigated lands are affected with salinity [5]. About one third of all irrigated land is considered to be affected by salt due to the secondary salinization [6] and it is estimated that about 50% of the arable land is likely to be salinized by 2050. In the coming years, as the quality of irrigation water continues to decline, the problem due to salinity will become ever more acute.

Over the next three decades, production of food grains in India has to increase at least two million tonnes a year to meet the food demand of the burgeoning population. And this increase has to come from vertical increase in yields from major crops grown since scope of horizontal expansion of cultivable land is virtually nil. One practical means to achieve greater yield is to minimize the abiotic stress associated losses. Attempts to improve yield under stress conditions through conventional plant breeding have been by and large remain unsuccessful, primarily due to the multigenic origin of the adaptive responses. Integration of transgene through genetic engineering / rDNA technology of crop plants appears more feasible. With the advent of genetic transformation techniques based on rDNA technology, it is now possible to dispense alien genes into a recipient plant genome that confers tolerance to abiotic stress including excess salinity/NaCl. Microprojectile bombardment is an important tool in developing transgenic plants especially when the plants are found to be recalcitrant to *Agrobacterium*-mediated transformation [7,8,9]. Ability to deliver foreign DNA directly into regenerable cells, tissues, or organs is one of the best methods to develop transgenic lines based on independent transformation events in many agronomic crops through bypassing *Agrobacterium* host specificity and tissue culture-related regeneration difficulties. Absence of biological constraints makes particle bombardment based methodology a versatile and effective transformation method best suited for the genetic manipulation of important crops, not limited by cell type, species or genotype. Keeping this in purview, it is imperative to deploy genetic transformation techniques to meet the future challenges for increased productivity.

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II.2. BIOTIC STRESS

Among different phyla in the animal kingdom, insects occupy a substantial portion of the biota canvas and displays maximum diversity in comparison to others. Their fossils that are found in the carboniferous period of the geological era that is about 250 million years old indicate their ancient nature. With estimated 5 million species of insects, even to provide a scientific name is a herculean task for the insect taxonomists in contrast to the capability of vertebrate taxonomists, who work with smaller and better-known taxa. Elaborate discussion about insect biodiversity does not come under the purview of this article; however, it is prudent to mention that diversity even in agricultural crop pests is fabulous within the overall ambit of insect biodiversity as evident across different continents over diverse crop plants. Insect pest problems in agriculture are probably as old as agriculture itself. Following the introduction of high yielding varieties (HYVs) and associated technology, there has been a tremendous increase in the number of insect pests damaging various crops. The extent of damage is influx and a large amount is being spent every year for purchase and application of insecticides in implementing biological control or in adopting modern strategies. The yield is also limited owing to synergistic effect of abiotic along with biotic stresses, wherein understanding the molecular basis of such interactions becomes imperative to unzip the molecular and physiological responses and holds key factor in determining the stress-tolerance strategies which can be adopted [12]. Genetic transformation is one such technique i.e., dove-tailing foreign gene(s) within the genome of plants in productive background efficiently and effectively, which holds immense potential to develop broad-spectrum stress-tolerant plants to avert the impending doom of stress to obtain robust and high-yielding varieties of crop plants [1].

III. TRANSGENIC DEVELOPMENT

The importance of genetic transformation in dispensing foreign gene(s) into a desired genetic background in order to modify and agronomically improve upon the crop plants can hardly be over emphasized. It is important because it precisely transfers desired alien gene/s from homo/heterologous sources to a recipient system directly or indirectly through bacterial or viral mediation or through microprojectile based bombardment using DNA coated gold microcarriers acting to move a specific piece of DNA into the protoplasts or intact cells/ tissues. Spectacular advancement has been made in crop genetic modulation through genetic transformation, especially for those characters, which are not found to be amenable through conventional breeding. The transfer of a specific gene or multigenes into a recipient genome has wider application in the genetic manipulation of crop plants. Ideally, a successful plant genetic transformation involves the transfer of foreign gene(s) into a recipient system with its subsequent expression and inheritance of the new DNA in the progenies. Gene transfer methods could be primarily classified as i) vector less system, which notably includes direct transfer of naked DNA through particle bombardment or electroporation of genes through membrane permeabilisation using high molecular weight polyethylene glycol (PEG) and ii) vector based system such as bacteria, viruses, fungi etc. Among various gene transfer methods, of late, *Agrobacterium*-mediated gene transfer system is gaining preference in rice due to its low cost, convenience and high probability of single copy gene integration with minimum rearrangements [13]. In rice, genotype specificity of the cultivar and strain specificity of *Agrobacterium* seriously hinders the transformation efficiency. Thus, it becomes essential to evolve a race, genotype and strain independent efficient transformation system. Successful developments of transgenics were reported for a wide range of crops like maize [14], oilseed rape [15], potato [16], soyabean [17], cotton [18], tomato [19] etc.

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Table (1): Some major genes harbouring potential application for abiotic and biotic stress remediation via transgenic research. Source: Roy *et al.* 2011; Atkinson and Urwin, 2012.

GENE	SPECIES	MECHANISM OF ACTION	REFERENCES
Abiotic			
Salt stress tolerance			
DREB	<i>Arabidopsis thaliana</i>	Enhanced tolerance to salinity, cold and drought	Kasuga <i>et al.</i> (1999)
ApoInv	Tobacco	Salt tolerance to high osmotic pressure increase in cell sap	Fukushima <i>et al.</i> (2001)
Osmotin	<i>Oryzasativa</i>	Enhanced salt and drought tolerance	Barthakure <i>et al.</i> (2001)
pgAHX1	<i>Pennisetum glaucum</i>	Enhanced salt tolerance	Rajgopala <i>et al.</i> (2007)
MnSOD	Poplar	Enhanced salt tolerance	Wang <i>et al.</i> (2010)
Water logging tolerance			
pdc1	Rice	Enhanced submergence tolerance	Qunimio <i>et al.</i> (2000)
ADH	Cotton	Enhanced ethanol fermentation	Dennis <i>et al.</i> (2000)
ACC	Tobacco	Enhanced flooding tolerance	Grichko and Glick (2001)
Sub1A	Rice	Submergence tolerance	Xue <i>et al.</i> (2006); Fukao and Bailey-Serres (2008)
HRE1	<i>Arabidopsis thaliana</i>	Improve the resistance by enhancing anaerobic gene expression and ethanolic fermentation	Licausi <i>et al.</i> (2010)
HRE2			
Drought tolerance			
Sod	<i>Medicago sativa</i>	Enhanced tolerance to water deficit	McKersie <i>et al.</i> (1993)
Adc	<i>Oryzasativa</i>	Reduced chlorophyll loss under stress	Capellet <i>et al.</i> (1998)
HVA1	Pusa Basmati 1	Enhanced stress tolerance in terms of cell integration and growth	Rohila <i>et al.</i> (2002)
NPK1	<i>Solanum tabaccum</i>	Enhanced drought tolerance	Shoue <i>et al.</i> (2004)
ABF3	<i>Arabidopsis thaliana</i>	Enhanced drought tolerance	Abdeen <i>et al.</i> (2010)
Heat tolerance			
Hsp70	<i>Arabidopsis thaliana</i>	Enhanced thermotolerance in transgenic plants	Lee and Schoff (1996)
Hsp17.7	<i>Daccus carota L.</i>	Enhanced or decreased thermotolerance	Malik <i>et al.</i> (1999)
PSCR	<i>Glycine max</i>	Enhanced proline accumulation	De Ronde <i>et al.</i> (2000)
Hsp101	<i>Arabidopsis thaliana</i>	Manipulated thermotolerance	Queitsch <i>et al.</i> (2000)
DcHSP17.7	<i>Solanum tuberosum L.</i>	Improved cellular membrane stability and enhanced <i>in vitro</i> tuberization	Ahn and Zimmerman (2006)
Cold tolerance			
Soc1	Alfalfa	Enhanced tolerance to freezing stress	McKersie <i>et al.</i> (1993)
Cod A	<i>Arabidopsis thaliana</i>	Seedling tolerant of salinity stress and Enhanced germination under cold	Hayashi <i>et al.</i> (1997)

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AB13	<i>Arabidopsis thaliana</i>	Enhanced freezing tolerance	Tamminen <i>et al.</i> (2001)
Gs2	Rice	Enhanced salinity resistance and chilling tolerance	Hoshida <i>et al.</i> (2000)
atRZ-1a	<i>Arabidopsis thaliana</i>	More tolerant to cold	Kim and hunseung (2006)
Metal toxicity tolerance			
MsFer	<i>Nicotianatabacum</i>	Enhanced tolerance of oxidative stress caused by excess Fe	Deake <i>et al.</i> (1999)
par β	<i>Oryzasativa</i>	Tolerant to higher concentration of Al-toxicity	Conway and Toennissen (1999)
arsC	<i>Nicotianatabacum</i>	Significantly greater Cd tolerance	Dhankher <i>et al.</i> (2003)
ALS3	<i>Arabidopsis thaliana</i>	Aluminum tolerance	Larsen <i>et al.</i> (2005)
ALMT1	<i>Arabidopsis thaliana</i>	Aluminum tolerance	Hoekenga <i>et al.</i> (2006)
Biotic			
BOS1	<i>Arabidopsis thaliana</i>	Restricts spread of necrotrophic pathogens and provides tolerance to abiotic stresses	Mengisteet <i>al.</i> (2003)
MYB4	Rice	Imparts resistance to abiotic (cold, drought, salt, ozone) and biotic stresses (viruses, bacteria, fungi)	Vannini <i>et al.</i> (2004, 2006, 2007)
AIM1	Tomato	Integrates responses to biotic and abiotic stresses by modulating ABA signaling and ion fluxes	AbuQamareet <i>al.</i> (2009)
PIMP1	Wheat	Modulates response to biotic and abiotic stresses	H.X. Liu <i>et al.</i> (2011)
ATAF1	<i>Arabidopsis thaliana</i>	Abscisic acid-inducible factor that represses abscisic acid signaling during pathogen infection	Jensen <i>et al.</i> (2008)
ATAF2	<i>Arabidopsis thaliana</i>	Integrates wounding and pathogen defence responses	Delessert <i>et al.</i> (2005)
NTL6	<i>Arabidopsis thaliana</i>	A cold-activated inducer of PR gene expression and disease resistance	Seo <i>et al.</i> (2010)
NAC6	Rice	An inducer of biotic and abiotic stress responses that has a negative effect on growth	Nakashima <i>et al.</i> (2007)
NAC4	Wheat	A transcriptional activator in biotic and abiotic signaling pathways	Xia <i>et al.</i> (2010)
DEAR1	<i>Arabidopsis thaliana</i>	Negatively regulates components of the salicylic acid-mediated pathogen response	Tsutsui <i>et al.</i> (2009)
ERFLP1	Hot pepper	Targets binding domains in genes involved in pathogen defence and salt tolerance	Lee <i>et al.</i> (2004)
BIERF1-4	Rice	Positive regulators of disease resistance responses that may also regulate tolerance to abiotic stresses	Cao <i>et al.</i> (2006)
ERF3	Soybean	A positive regulator of defence and abiotic stress genes, causing biotic and abiotic stress tolerance when expressed in tobacco	G.Y. Zhang <i>et al.</i> (2009)
TS1	Tobacco	Positive regulator of genes in salt tolerance and PR gene-associated pathogen defence pathways	Park <i>et al.</i> (2001)

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OPBP1	Tobacco	Overexpression gives tolerance to fungal, bacterial, and salt stress	Guo <i>et al.</i> (2004)
AREB1	Tomato	Functions in abscisic acid-mediated abiotic stress and pathogen response signaling	Orellana <i>et al.</i> (2010)
WRKY45	Rice	Increases pathogen resistance in rice and Arabidopsis when overexpressed, as well as inducing abscisic acid- responsive abiotic stress tolerance genes	Shimono <i>et al.</i> (2007); Qiu and Yu (2009)
WRKY82	Rice	Regulates responses to biotic and abiotic stresses through the jasmonate/ethylene signaling pathway	Pen <i>et al.</i> (2011)
ZAT7	<i>Arabidopsis thaliana</i>	Suppresses a repressor of defence responses to a wide range of stresses, including WRKY70	Rizhsky <i>et al.</i> (2004a); Ciftci-Yilmaz <i>et al.</i> (2007)
HSFA1B	<i>Arabidopsis thaliana</i>	Regulates basal resistance to biotic and abiotic stresses and maintains growth rate under stress	Mullineaux <i>et al.</i> (2011)

In today's agriculture especially in developed countries transgenic technology holds out as a highly promising area. About 25,000 field trials have been conducted involving 60 species and 10 characters in 45 countries. Now transgenic plants are available, which can withstand biotic stresses like weeds, insects, disease-causing pathogens, viruses, nematodes and abiotic stresses like drought, salinity, alkalinity, oxidative stress, frost and cold, contain improved nutritional qualities like altered fatty acid composition, changed amino acid quantity, delayed fruit ripening, altered flower pigmentation, male sterility, dwarfism, increased photosynthesis efficiency etc [20]. India's joining in growing GM crop for the first time in 2002, the three most populous countries in Asia-China, India and Indonesia accounting for almost 2.5 billion populations are now all set to venture into commercializing GM crops. Also, we cannot increase the area of land under cultivation in order to ensure containing undesirable erosion of natural habitats; the productivity of the current area of cultivated land will have to be enhanced. Transgenic crops are likely to play an increasingly important role in world of agriculture in the coming decades. To feed the ever-increasing population more and more food needs to be produced from dwindling resources like less land, water and energy and declining bio resources by increasing the productivity of agricultural crops to considerable extent.

An eco-friendly approach in management of insect pest is the use of soil bacterium *Bacillus thuringiensis*, which has attracted attention of both microbiologists and entomologists since long back. Use of spores from naturally occurring *B. thuringiensis* in the fermented forms was found to be promising in controlling insects. The first *B. thuringiensis* strain was commercialized and marketed as 'thuricide', which were used as spray and in the form of aerosols. This free-living bacterium is abundant in the soil environment and different strains are available worldwide. Bt proteins possess specific insecticidal action towards certain insect orders and are reported to be safe to non-target insects, birds and mammals. Delta (δ) -endotoxins possesses two properties that are essential for toxins used in transgenic crops. These are highly toxic to certain insect pests, and reported to be safe for human consumption [21]. Upon ingestion by susceptible host, endospore sporulates and produces an array of toxins, including crystalline delta (δ) toxin. In presence of proteases and at high pH of 7.5-8.0 in insect gut environment, the protoxins (65-70 kDa) cleave to yield an active toxin, which percolates through the peritrophic membrane and bind to specific receptors on the apical microvillar brush border membrane. In highly sensitive insect species, the microvilli lose their characteristic structure within minutes of insertion and the cells become vacuolated and ultimately burst. The alkaline gut juice begins to leak out into the haemocoel. Consequently, the haemocoel pH rises causing paralysis, which leads to inhibition in feeding and eventual death of the insect [22]. Based on structure, antigenic properties and activity spectrum, crystal proteins and their genes have been classified into four major groups: cry I (Lepidoptera specific), cry II (Lepidoptera and Diptera specific), cry III (Coleoptera specific) and cry IV (Diptera specific). The Lepidopteran-active cryI genes are the most thoroughly studied group among all δ -endotoxin genes. The cry I genes encode proteinaceous protoxins of 130-140 kDa, which were found to be localized within bacterial spores as bipyramidal crystal inclusions. The δ -endotoxins and associated peptides remain present in less quantities, however, may super code amounts greater than 20% of the weight of the spore [23]. Despite the unprecedented success Bt products accounts only about 1 % of the total 'agrochemical market'.

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Use of Bt based products remained largely restricted almost exclusively to high value or environmentally sensitive niche markets as fresh market vegetables owing to several factors. One notable limiting factor is its narrow spectrum of insecticidal activity. A high degree of target specificity obviously makes Bt a desirable alternative to broad-spectrum chemical insecticides from environmental standpoint, but also limits its market potential. Bt sprays also have limited field efficiency since they may be washed off, by rain and are found to be prone to degradation by heating effect under scorching sun. This often necessitates recurrent applications, which is expensive. Moreover, the conventional sprays are found to be inaccessible to the internal tissue feeders like stem borers. These drawbacks of Bt sprays do not hold good for transgenic plants harbouring Bt genes. Transgenic Bt-plants translate required protein that acts as internal insecticides towards damaging insects, which feeds on plants. Considerable potential for genetic engineering of plants to express introduced Bt δ -endotoxin genes is looming large and substantial amount of work has been done in this context. With spectacular progress made in the techniques to engineer crops genetically, the ability to make broader use of 'natural' insecticides *in planta* has increased substantially. Development of tolerant lines towards insect pests through genetic management offers a cheap, more permanent and eco-compatible route in combating insect damage. Isolating Bt insecticidal genes from the original bacterium and introducing them into the plant genome allows crop protection from insect attack without repeating of external application of synthetic pesticides. The added advantage of engineered insect resistance in plants includes greater stability of δ -endotoxin proteins and independence in the time of application for effective control of the pest. Finally, protection is possible for the entire cropping season and would be exposed to only those insects, which would feed on genetically modified (GM) crops. The use of genetically engineered plants for deployment of resistance to insect pests has been reported to be eco-compatible. Large-scale cultivation of GM crops would be economic since the price of crop protection chemicals are hiking at an alarming rate. Furthermore in developing new varieties conventional breeding progress has been found to be slow, tedious and often misfires. Plant biotechnology has come up with more precision, ease and confidence in genetic manipulation of those characters, which are not amenable through conventional breeding. The main advantage of using Bt formulations as bio-insecticides is that such formulations are harmless to humans, mammals and to non-target fauna. The first commercial product, Sporeine, was available in 1938 in France [24]. Commercial Bt formulations include wettable powders, suspension, concentrates, water dispersible granules, oil miscible suspensions, capsule suspensions and granules [25, 26].

Plants are usually transformed with relatively simple constructs, in which the gene of interest is fused to a promoter of plant, viral or bacterial origin. Generally ideal constructs contain a 5' promoter, a multiple cloning site with a number of unique restriction endonuclease cleavage sites (transgene remains housed in this site), and 3' transcriptional terminator, origin of replication sequence, reporter gene and selectable marker gene. Identification of suitable promoter to express high and constant levels of insecticidal protein to maintain stable effective resistance against target pests during the whole growing season is of paramount importance. Addition of introns was found to enhance the expression of genes in cereals [27]. Currently available constructs for genetic transformation of plants have largely been constructed from wild plasmids by genetic engineering. The cauliflower mosaic virus (CaMV) 35S RNA promoter is often used because it directs high level of expression in majority of the plant tissues. Other promoters such as the maize ubiquitinI promoter and the rice actin promoter are also been preferred in monocots. Besides constitutive promoter, tissue specific promoters are nowadays available. These promoters work stringently in specific tissues and thus help express specific gene/s in specific tissues, which is essential in many crops. Extensive research for identification of callus specific promoter is progressing in diverse laboratories worldwide. Selective markers either confer resistance to a selective agent (e.g. antibiotics or herbicides) or display enzymatic activity (e.g. β -glucuronidase or luciferase) in identification of true transformants. Use of selective agents particularly antibiotics is often critical in selecting transformants, which needs to be optimized for efficient development of transgenics. Sometimes additional regulatory sequences are also included with the gene and the promoter acts as transcriptional or translational fusion forms for hyper expression of transgene. The genes are generally introduced either as naked DNA or ligated into a plasmid, or cloned into specialized vector with required regulatory elements for dispensing through *Agrobacterium*. The coding sequences of the genes for bacterial enzymes which are easily assayed and whose activity is not normally found in plants constitute the basis of reporter genes. These sequences indirectly report the presence of transgene since it rests in the same plasmid construct generally. The GUS coding sequence (*gus*) is being used extensively used in plant genetic transformation as reporter

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gene, either as markers for monitoring transformation efficiency or for molecular characterization of regulatory elements isolated from a number of different plant genes [28].

IV. CONCLUSION

Although in the recent past, India has achieved self-sufficiency in food grain production, concerns of food security will remain as ever owing to the ever-increasing population of the country. To sustain the self-sufficiency and nutritional security in the ensuing decades, the production of rice needs to be increased every year by almost 2 million tons, which seems to be a stupendous task especially in the context of discernible plateauing trend as observed in yield levels of HYVs and at the interface of declining natural resources. An increase in the yield potential crops in fragile environments is a crucial need of the hour, for which research needs to be redefined to surmount the technological challenges in breaking the yield barrier, improvement in input use efficiency and development of environmentally accepted technology in which genetically modified varieties seem to be holding the key position. But the ability of cells to regenerate plantlets under *in vitro* culture varies largely. Such variations arise not only with the type of cells and tissues used but also among the genotypes within the same species. This causes a serious constraint in formulation of a generalized protocol for successful genetic transformation as in genetic transformation systems; the aim is to regenerate plants identical to the parent, except for the newly inserted genes and to obtain an uniform expression of the gene to avert the constraints associated with abiotic and biotic stress factors to obtain a safe and secure future of agriculture worldwide.

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