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# Bt brinjal in India

## A long way to go

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Brinjal or eggplant (*Solanum melongena*) is the major vegetable crop in India and is vulnerable to many diseases caused by insects, pests, fungi, bacteria and viruses. Brinjal production is extensively affected by the brinjal fruit and shoot borer (FSB, *Leucinodes orbonalis*). Use of conventional chemical pesticides not only damages the environment, including the biotic and abiotic components, but also affects human health. Bt Brinjal was developed to combat brinjal fruit and shoot borer that has an advantage, minimizing the use of chemical pesticides. Extensive biosafety investigations, nutritional studies, substantial equivalence studies, relative toxicity and allergenicity assessment using animal models like Sprague Dawley rats, Brown Norway rats, rabbit, fish, chicken, goats, etc., revealed no significant differences between genetically modified brinjal and its native counterpart. Bt brinjal effectively controlled the target pest and was found to be safe for environment and human health. In spite of all the scientific studies, release of Bt Brinjal has been placed under moratorium. The Indian government has constituted an expert committee to address this issue. In this review we have tried to explore the facts related to Bt Brinjal including its production, use of Bt toxin, use of chemical pesticides in controlling the FSB in native brinjal, along with the perspective of public opinion and government initiatives.

more than doubled in the last 20 years, increasing from 202,000 hectares in 1987 to 553,000 hectares in 2005. This increase in area along with increase in productivity has contributed to more than a 3-fold increase in production from 1987 (2.6 million tons) to 2005 (9.1 million tons).<sup>2</sup> Brinjal occupies the major position (Fig. 1) according to production amongst vegetable crops.<sup>3</sup> The major brinjal producing states are West Bengal, Orissa, Gujarat, Bihar, Maharashtra, Andhra Pradesh, Chhattisgarh, Madhya Pradesh, Karnataka, Asam, Haryana and Tamil Nadu. The area under brinjal cultivation is estimated at 0.512 million ha with production of 8.4 million metric tonnes in 2007.<sup>4</sup> The varieties of brinjal popular in India include, Arka Navneet, Pusa Ankur, Hybrid-6, Pusa Hybrid-5, ARBH-1, ABH-1, Pusa Purple Long, Pusa Purple Cluster and Ritu Raj. West Bengal is the largest producer of brinjal followed by Maharashtra and Bihar.<sup>5</sup> Brinjal accounts for 8.4% of total vegetable production and covers 8.14% of land under vegetable cultivation. It constitutes an important ingredient in ayurvedic medicine which is used in the treatment of diabetes and liver problems.<sup>6</sup> In India, a large number of brinjal varieties and hybrids are grown by small, marginal and resource poor farmers throughout the year. It is the major source of profitable income for farmers because of local demand, and it is a perennial crop so that fruit can be harvested throughout the year.

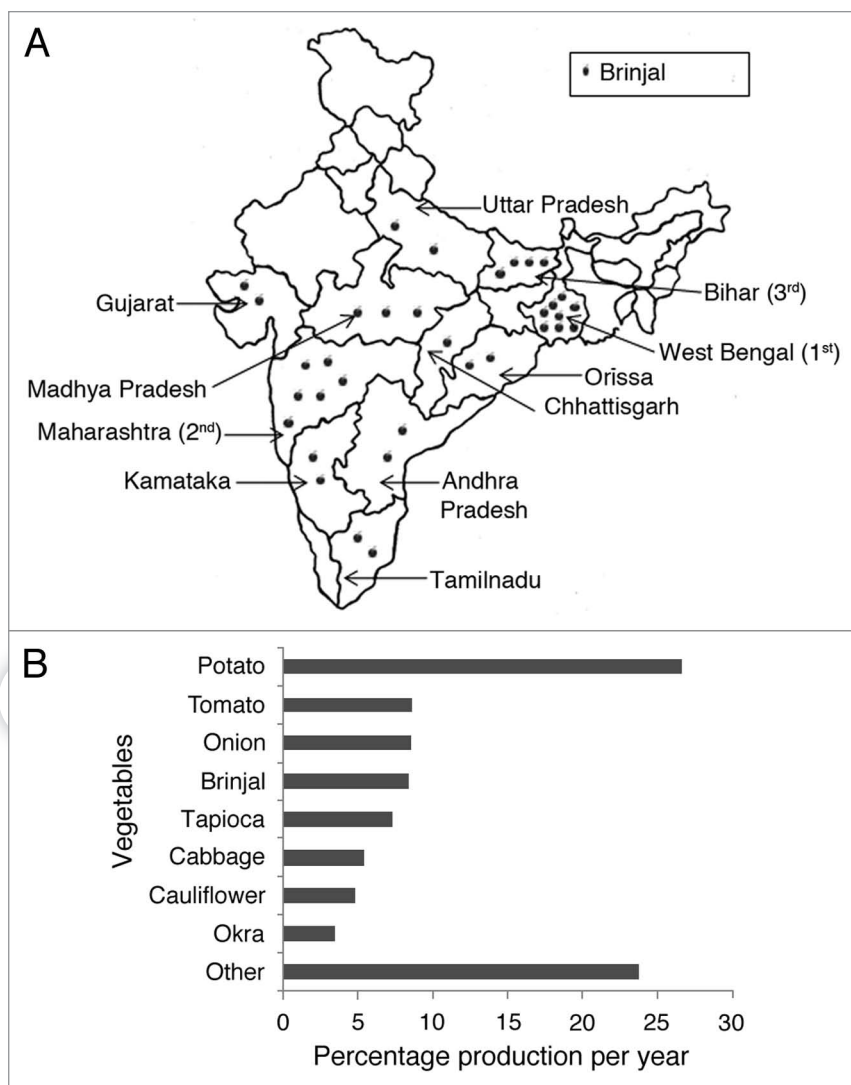
### Introduction

Agriculture is one of the most important sectors of the Indian economy. It is the means of livelihood of almost two thirds of the population of the country and according to the economic data for the financial year 2006–07, agriculture accounts for 18% of India's GDP.<sup>1</sup> About 43% of India's geographical area is involved in agricultural activity. Though the share of Indian agriculture in the GDP has steadily declined, it is still the single largest contributor to the GDP and plays a vital role in the overall socio-economic development of India. The area occupied by brinjal or eggplant (*Solanum melongena*, family solanaceae) has

### Need of Bt Brinjal in India

The growth from 1.7 million hectares of biotech crops in 1996 to 148 million hectares in 2010 is an unprecedented 87-fold increase, making biotech crops the fastest adopted crop technology in the history of modern agriculture.<sup>7</sup> Nearly half of the transgenic crop area is located in the US. Maize covers 25% of the global transgenic crop area. Insect-resistant crops, based on toxins from the bacterium *Bacillus thuringiensis* (Bt) are the second most planted commercial transgenic crops and about 13% of the global transgenic crops area is covered with *Bt* maize. Therefore, Bt maize is the most widely grown Bt crop. Brinjal is prone to attack by many damages caused by insects, pests, fungi, bacteria and viruses. Brinjal productions are consistently and extensively damaged by brinjal fruit and shoot borer (FSB; *Leucinodes orbonalis*) and other fruit borer insects *Helicoverpa arimegera*.<sup>8-11</sup> Brinjal FSB belongs to insect order Lepidoptera and family Crambidae. The distribution of FSB clearly indicates

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**Figure 1.** (A) Major Brinjal producing states in India. West Bengal is the largest producer of brinjal followed by Maharashtra and Bihar. (B) Production of vegetables including Brinjal. Vegetable production in India at the market level is as follows: 26.7% potato, 8.6% tomato, 8.4% brinjal, 7.3% tapioca (cassava), 5.4% cabbage, 4.8% cauliflower, 3.4% okra and 23.8% others.

that the pest causes damage to brinjal and is well established in major brinjal growing countries in Asia such as Bangladesh, China, India, Indonesia, Japan, Malaysia, Nepal, Pakistan, the Philippines, Sri Lanka, Taiwan and Vietnam. In Africa FSB is found in Ethiopia, Ghana, Kenya, Mozambique, Nigeria, South Africa, Tanzania, Uganda and Zimbabwe.<sup>2,12</sup> The pest successfully maintains high population due to its high reproductive potential and rapid turnover of generations. Damage symptoms include small darkened holes surrounded with brownish areas on the fruit surface and/or fruit stalk and wilted shoots. Fruit subsequently becomes hollow and filled with frass, therefore rendering the fruit unmarketable and refused by customers.<sup>13,14</sup> FSB is the most destructive and unmanageable pest of brinjal which accounts for the majority of insecticide use and major yield losses. Yield reductions can be as high as 70%.<sup>15,16</sup> Farmers also

have to apply additional insecticides to control other pests including epilachna beetle (hadda), stem borer, red spider mite and jassids. To protect brinjal from these insects, farmers use large quantities of chemical insecticides singly or in combination. Approximately, 25–80 sprays per season are applied for effective control of brinjal FSB.<sup>17</sup> This practice of indiscriminate use of insecticides leads to accumulation of pesticide residues in soil and ground water and ultimately causes environmental pollution. This excessive pesticide usage threatens the health of farmers and consumers, besides making eggplant fruit more costly to consumers. Gradually, the insect pest may also develop resistance to insecticide, exacerbating pest control. Several insecticides such as cypermethrin, endosulfan, deltamethrin fenvalerate, carbaryl, dichlorvos, phorate, carbofuran, lindane, fenitrothion, dimethoate and malathion are used to control FSB.<sup>18</sup> In addition, it remains challenging for researchers to develop superior cultivars that are resistant to insects and diseases by conventional breeding methods.<sup>19</sup> To reduce pest-linked damage in brinjal as well as to protect the environment from adverse effects of pesticides, deploying the lepidopteron-specific cry1Ac gene in brinjal would provide an effective built-in control for brinjal fruit and shoot borer as an insect resistance management strategy.<sup>20</sup> Some analysts are of the opinion that since the cultivation and consumption of brinjal in India is extensive; the commercialization of Bt brinjal will have significant economic implications for the farmers.

### Bt Protein: Better and Safer than Chemical Insecticides

*Bacillus thuringiensis* (*Bt*) is a Gram-positive soil bacterium. During its sporulation, crystalline inclusions are formed in the cells consist of different types of crystalline proteins (Cry proteins) with highly specific insecticidal activity. By the formation of an endospore, the sporangium lyses, and the proteins are released together with the endospore. The crystalline inclusions (protoxins) are not produced in a toxic form. Under specific conditions like high pH and the presence of specific proteases, the protoxins are transformed into an active form. When insects in the soil ingest these crystalline inclusions, the protoxins dissolve in the midgut of the insects, as the pH in the midgut is very high (about 10.5). At low pH the protoxins are insoluble. Dissolution of the crystalline inclusions releases crystalline proteins, which are cleaved enzymatically by specific proteases into active, toxic polypeptides.<sup>21</sup> Active toxins interact with the membranes of the midgut epithelium and build pores in the cell membranes.<sup>22</sup> The osmotic imbalance leads to swelling and lyses of the cell, therefore the insect dies.<sup>23</sup> Unlike

most of the chemical insecticides, Bt does not function on contact, rather it acts as a mid-gut toxin.<sup>24</sup> The Cry proteins from Bt crops are released into the soil by different pathways such as root exudates, pollen and harvest residues.<sup>25,26</sup>

The active toxin does not require a high pH for solubilization and no specific proteases are needed to activate the toxins. Cry proteins can be inactivated or removed from the soil environment by (1) consumption by insect larvae, (2) degradation and mineralization by micro organisms or (3) sunlight.<sup>27</sup>

To be active and function as an insecticide, Bt proteins require certain specific conditions. These include:

(1) Bt protein has to be ingested by the target insect.

(2) Bt protein requires an alkaline pH (9.5 and above) for its activation.

(3) Presence of specific receptors particularly cadherin, in the insect mid-gut epithelial cells is required for protein binding.

FSB larvae that attack brinjal plants possess all these above mentioned specific conditions and therefore die when they feed on Bt brinjal. Bt brinjal does no harm to non-target organisms due to absence of specific receptors and conditions to activate Bt protein in their gut. In addition, studies of the US Environmental Protection Agency (US-EPA) showed no differences in the populations of microbes in soils that contained Bt plant material or conventional plant material or in the populations of soil microorganisms, and plant pathogens between fields of Bt and non-Bt crops.<sup>28,29</sup> Several studies show that Bt proteins do not persist in the soil and are broken down in a matter of weeks.<sup>30-32</sup> Bt technology may be considered a superior technology for control of target pests because of its property of target specificity and less prone to resistance development.

### How was Bt Brinjal Created?

Bt Brinjal is the first genetically modified vegetable crop in India that has reached the approval stage for commercialization and will be directly consumed by humans, if released. Bt Brinjal is generated by inserting a gene *cry1Ac* from a naturally occurring soil bacterium called *Bacillus thuringiensis* which has been used for a long time as a biopesticide in horticulture.<sup>33</sup> Transgenic brinjal was developed by the Maharashtra Hybrid Seed Company Ltd., (Mahyco), a leading Indian seed company; the Tamil Nadu Agricultural University, Coimbatore, the University of Agriculture in Dharwad (Karnataka) and two Indian Councils of Agricultural Research (ICAR) laboratories are also involved in developing genetically modified versions of brinjal. The insertion of the gene into the Brinjal cell in young cotyledons has been done through an Agrobacterium-mediated vector, along with other genes like virus (CaMV) 35S promoters, *nptII* gene encodes the selectable marker enzyme neomycin phosphotransferase II (NPTII) as markers and *aad* gene 3''(9)-O-aminoglycoside adenyl transferase (AAD) allowed for the selection of bacteria containing the pMON 10,518 plasmid on media containing spectinomycin or streptomycin. The *cry1Ac* gene along with two other supporting genes namely *nptII* and *aad* genes are put together in such a way that they work in tandem to produce insecticidal protein that is toxic to the targeted

insect, in this case the fruit and shoot borer. NPTII and AAD proteins are used as a selectable marker and have no pesticidal activity and are not known to be toxic to any species.<sup>34,35</sup>

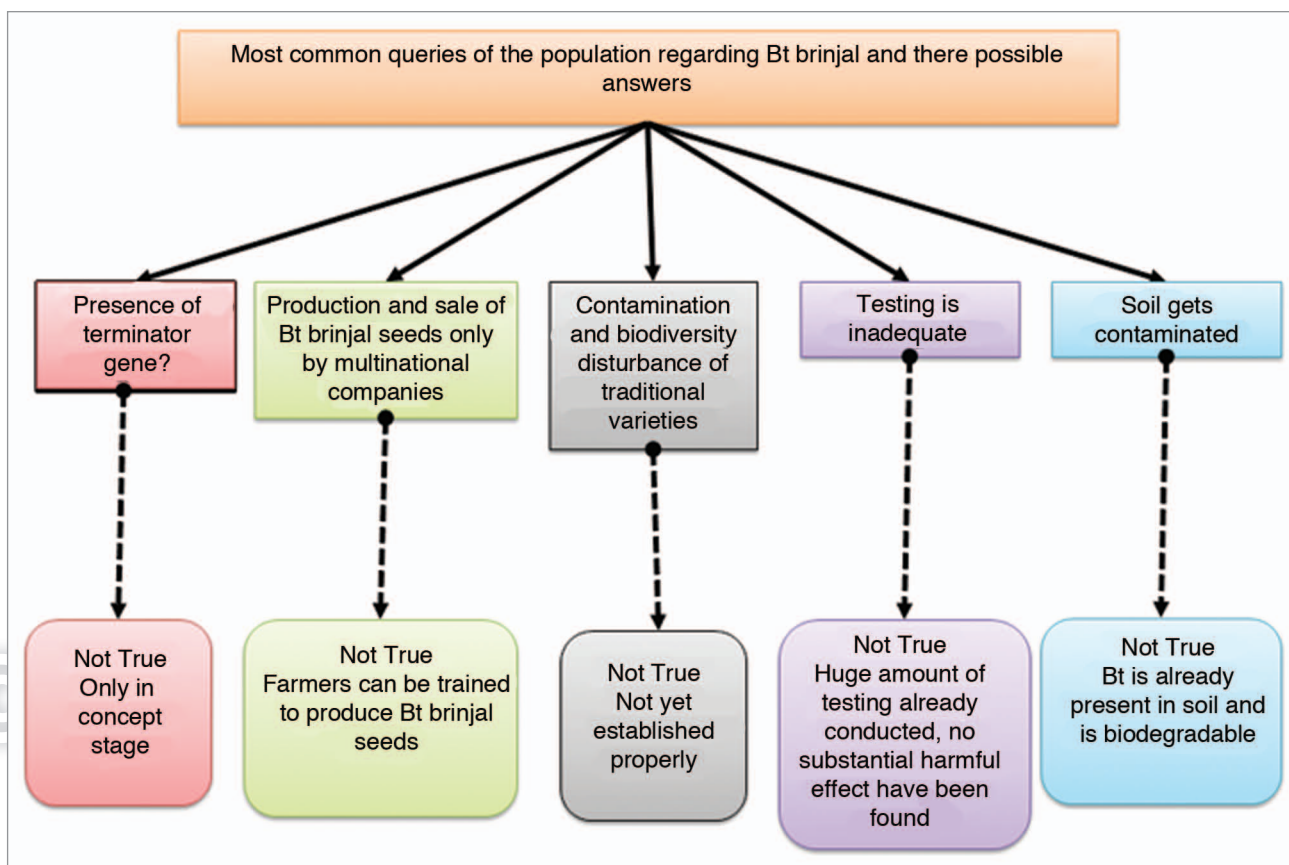
### Bt Brinjal Development and Safety Studies

Since 2000, the tale of creation of Bt Brinjal was started. Greenhouse evaluation of Bt brinjal was evaluated in 2000–01.<sup>36</sup> Their pollen flow study at different locations was observed and backcrossing program initiated in 2002.<sup>37</sup> Acute oral toxicity studies in rats, mucous membrane irritation test in female rabbit and primary skin irritation test in rabbit were assessed by Intox, Pune in 2003.<sup>38</sup> Multilocation field trials of five hybrids (MHB-4, 9, 10, 80 and 99) and effects on non-target and beneficial insects were studied in 2004.<sup>39,40</sup> Sub chronic oral toxicity study in Sprague Dawley rats (Intox, Pune), assessment of allergenicity of protein extracts using Brown Norway rats (Rallis, Bangalore), responses as a dietary feed ingredient to common carp (*Cyprinus carpio*) growth performances (Central Institute of Fisheries Education, Mumbai) and ICAR second year trials for five hybrids (MHB-4, 9, 10, 80 and 99) was performed during 2005.<sup>41-43</sup> Chemical fingerprinting of Bt and non-Bt brinjal (including alkaloids) (Indian Institute of Chemical Technology, Hyderabad), sub chronic (90 days) feeding studies using New Zealand rabbit (Advinus Therapeutic, Bangalore), effect on performance and health of broiler chickens (Central Avian Research Institute, Izatnagar), subchronic (90 days) feeding studies in Goats (Advinus Therapeutic, Bangalore), feeding studies in lactating crossbred dairy cows (G.B. Pant University of Agriculture and Technology, Pantnagar) and socioeconomic and risk assessment studies have been done.<sup>44,45</sup> Large scale field trials were conducted by the Indian Institute of Vegetable Research to evaluate agronomic performance and environmental impact of Bt brinjal hybrids during 2007–09.<sup>46</sup> Several other studies include germination and weediness studies, aggressiveness studies, soil microbiota studies (two years), substantial equivalence studies, protein expression studies, baseline susceptibility studies (two years with 29 populations), food cooking and protein estimation in cooked fruits, molecular characterization were performed during 2008–09.<sup>47</sup>

Maharashtra Hybrid Seeds Company Mahyco is the leading name in the development of Bt Brinjal. In addition to Mahyco, the National Research Center for Biotechnology at the Indian Agricultural Research Institute (IARI) is also experimenting with Bt Brinjal. No significant differences were noted between Bt Brinjal and Non-Bt Brinjal, in studies such as acute oral toxicity, sub-chronic oral toxicity in rats and allergenicity of protein to rats, germination, weediness and aggressiveness tests and soil micro-biota studies.<sup>47</sup>

The University of Agricultural Sciences (UAS), Dharwad, developed six varieties of Bt and all six varieties have undergone field trials for their agronomic performance. The effectiveness of Bt gene for controlling the FSB has also been tested. Bt brinjal was found effective in controlling the target pest that means expression of *cry1Ac* protein was adequate to kill the FSB. Additionally, it was safe for other organisms such as soil microbes, nematodes,





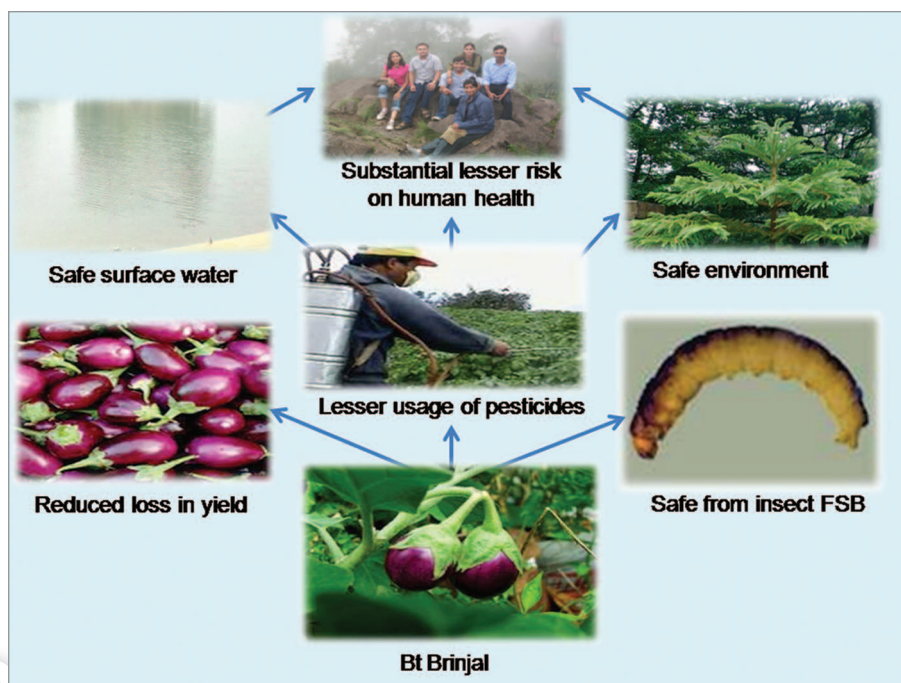
**Figure 2.** Most common queries of the population and their appropriate answers.

earthworm, etc. To assess the safety and toxicity of Bt brinjal, different tests have been carried out at Mahyco Research Center, Dawalwadi (Maharashtra). These studies include environment and biosafety investigation, nutritional studies, substantial equivalence studies, relative toxicity and allergenicity assessment using animal models like Sprague Dawley rats, Brown Norway rats, rabbit, fish, chicken and goats. All the bio-safety studies conducted have shown that there were no significant differences between Bt and non-Bt brinjal.<sup>47</sup> In conclusion, Bt brinjal effectively controlled the target pest and was found safe for environment and human health.

### Controversies and Arguments for the Release of Bt Brinjal

Since 1996, when GM foods introduced to world markets like GM soya, corn, canola and cotton seed oil, safety is the main concern about these GMs and are a big time issue. India has denied the entry of first genetically modified vegetable Bt brinjal in to the market and put it on hold for “indefinite time” because people have many “doubts” in their mind (Fig. 2). This is not a new story behind the introduction of GM foods into the market. Earlier in the case of Bt maize, questions were raised regarding the risk to the monarch butterfly by consumption of Bt corn pollen, that was found to be inappropriate.<sup>48,49</sup> The issue of cross contamination has also been raised against Bt maize, but it was rarely

found between maize and grasses. Stunted growth of the kidney due to consumption of GM foods has also been mentioned, but the European Food Safety Authority (EFSA), after re-examining the safety data on corn, concluded that the observed small size was not due to Bt maize feeding. Fear of Monsanto controlling our food chain has also been raised if Bt brinjal is approved since Mahyco is involved in development of a hybrid Bt brinjal (26% Mahyco is owned by Monsanto itself) but two Indian universities, Tamilnadu Agricultural University, Coimbatore and the University of Agricultural Sciences, Dharwad have also participated and developed Bt brinjal varieties which may dispel the myth of a Monsanto monopoly. Bt cotton is not comparable to Bt brinjal without a doubt, but it is nevertheless necessary to review our experience with it. Bt cotton has put India into a good position in the world as far as cotton production is concerned, after new technology took root. Resistance development is a very serious concern for monophagous pests, so there is need to develop baseline susceptibility data of cry toxin on the fruits and shoot borer population from all the brinjal growing regions.<sup>50</sup> Most of the data available about Bt brinjal are available from Mahyco, therefore there is also a need to set up a main resistance monitoring laboratory to assess the changes in baseline susceptibility changes of the fruit borer to cry proteins after releasing the technology. As India is at the center of the origin of cultivated brinjal, transgenes can move to the wild germplasm through this and we will not be able to differentiate between Bt brinjal and non-Bt



**Figure 3.** The major benefits of Bt Brinjal. The major benefit of use of Bt brinjal are safety from insect Fruit and shoot borer (FSB), reduced loss of yield, lesser usage of pesticides, safe surface water, safe environment and substantially lesser risk on human health.

brinjal, making labeling impossible. Indian systems of medicine, including Ayurveda, Sidha, Homeopathy and Unani use brinjal as a medicinal ingredient, both in raw and cooked form, for treatment of respiratory diseases and the entire brinjal plant is used in such preparations. There is fear that Bt brinjal will destroy these medicinal properties due to loss of synergy, differences in alkaloids and changes in other active principle. Some in the Indian population strictly oppose the release of Bt brinjal. The main cause of the Bt brinjal controversy was use of the Bt gene. People believe the use of the Bt gene may be harmful as there is no full proof safety that has been claimed by scientists. In addition, people also think that unlike GM cotton it is a food that is daily consumed by human beings. Some of the scientists have raised three very important issues:

- (1) Data on testing of chronic toxicity,
- (2) Independent tests that command credibility and don't only depend on data provided by the developers themselves and,
- (3) The need to have an independent regulatory system that will be in a position to study all aspects of GM technology in agriculture.

Consideration is ongoing over release of Bt brinjal in limited areas and ensuring that its sale would be monitored through mandatory labeling. However, labeling will be impractical in India because the retail market is fundamentally different than that of developed countries and also due to its difficulty in monitoring limited usage in practice.

Bt brinjal is the first GM food crop under evaluation for commercial release in India and also will be the first GM brinjal to be released globally. GM brinjal is currently not approved in India due to differences of opinions about Bt brinjal in the populace.<sup>51-54</sup> The government of India recently announced that Bt

brinjal needs additional time for review. In view of controversies over Bt Brinjal, the Genetic Engineering Approval Committee (GEAC) decided to set up an 'Expert Committee' on Bt brinjal in 2006. In October 2009, GEAC declared Bt Brinjal safe and recommended its commercial approval to the environmental ministry who subsequently imposed a moratorium on commercial release of Bt Brinjal.<sup>21</sup> An independent joint panel of India's GEAC and eminent scientists on gene technology favor lifting of the moratorium and allowing limited release of Bt Brinjal under strict monitoring during the first meeting of the expert panel held in April 2011.<sup>55</sup>

## Conclusions

Biotechnology, particularly genetic modification of crop plants may be one of several tools that can be extremely helpful in fulfilling the need to increase food production, nutrition and global food security. Specifically, production of Bt brinjal may considerably reduce the cost while also minimizing the risk of negative health impacts to those applying insecticides (Fig. 3). Now the question arises "should there be some more tests?" If yes, it should be fast tracked. Once the doubts are addressed, release of Bt brinjal needs to be encouraged in India without further delay, as several countries have approved and are consuming GM foods without any problems. Final approval and release of Bt brinjal will benefit not only the producers of the crop, i.e., the farmers, it will also help grow the Indian economy. It will also result in reduced pesticide residues in soil and water, less air pollution and local environmental pollution due to decreased use of insecticides, protection of naturally occurring predators and parasitoids and other beneficial organisms due to reduced use of

insecticides, reduction in soil and ground-water contamination, and safeguard soil microflora and invertebrates from damage. After extensive field trials, safety and adverse reaction studies, Bt brinjal was found to be safe for humans as well as the environment. According to regulatory authorities inadequate studies for the safety assessment of Bt brinjal need to be addressed including human trials, but here the required studies should be made clear so that the public can have pesticide free brinjal as soon as possible.

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