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印度转基因作物

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摘　要　印度政府于 2002 年批准转基因棉花商业化种植。截至 2014 年,单价或双价的转基因棉在印度大约占棉花种植面积的 95%。Bollgard 和 BollgardⅡ两种转基因棉花的推广对整个印度的棉花产业带来了巨大的影响,给农场、农民乃至国家都带来了利益。虽然印度转基因棉花取得了巨大成功,然而其他转基因作物在印度并没有实现商业化种植。本文就当前印度一些新的转基因作物研究进展、政府对转基因作物的监管及面临的挑战进行论述。

关键词　转基因作物; 转基因作物的监管; 印度

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印度在绿色革命后粮食安全及自给自足方面取得长足的进步,然而绿色革命带来的粮食增产现在却进入平台期。最新数据显示,2012－2013 年度和 2013－2014 年度粮食产量徘徊不前(图 1)。数据表明粮食增长速度正在减缓,然而国家对粮食增产的需求却依然存在。同时,民众对食品种类多样化的需求不断提高,需要一个侧重于种植蔬菜、豆类和水果等多样化的农业种植体系,以增加农民收入。随着印度人口不断增长、气候变化以及自然资源趋于枯竭,我们需要继续提高包括水稻和小麦在内的多种农作物的产量,以保证粮食安全。

生物技术的高速发展及其在农业生产上的应用,已使农民乃至消费者从中受益。目前各种农作物的损失主要来自于虫害、病害以及多种非生物逆境,这些都迫切需要通过生物技术来减少各种损失。抗虫转基因棉已获得了巨大的成功,我们也需要看到其他正处于研发中的携带优良性状的转基因作物尽早出现在农民的田地里。分子育种技术正广泛应用于一些经济作物,培育了一批优质的种质资源和产品。印度拥有一个强大健全的监管体系,多种携带优良性状如抗虫、抗除草剂、肥料高效利用、抗高盐、抗旱、抗病等的作物如秋葵、西红柿、卷心菜、菜花、玉米、大米、小麦、鹰嘴豆、橡胶都正处于不同的审批阶段中。

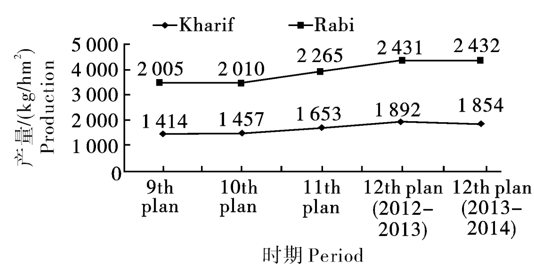

图 1　IX,X,XI 计划和 2012－2013 及 2013－2014 年度谷物的产量(Kharif & Rabi)

Fig. 1　Productivity of food grains in kg/hm^2 (Kharif & Rabi) over IX,X,XI Plans and 2012－2013 & 2013－2014

1　第一个转基因农作物

转 Bt 抗虫棉花品种已于 2002 年在印度成功获得商业化种植许可。双价基因聚合品种也于 2006 年获得商业化种植许可。随后便是多种其他作物诸如芥菜、马铃薯、茄子、秋葵、大米的产品正接受审核。然而,2010 年转基因茄子被环境和林业部叫停,这标志着一个客观的、以科学为基础的严格的监管体系演变成一个主观的、非科学驱动的政治决策。

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2 印度的生物技术监管

印度对转基因生物(GMOs)和rDNA产品的监管程序,已在1986年环境(保护)法案(EPA)和1989年条例中规定。该专门条例对有害微生物产品、基因工程产品或细胞,及在生产、使用/进出口、存储环节作了规定,该法案于1993年生效。一组关于重组DNA的准则于1990年发布,对基因工程生物、动植物的遗传转化、落实生物安全准则的机构和3种高危人群的防护措施等内容作了规定。为适应科学技术发展,1994年这个准则被修订为"生物技术安全准则修订版"。1998年,针对转基因植物提供专项审查,"转基因植物研究准则和转基因种子毒性致敏性评估准则(植物及植物部分)"生效。为适应转基因技术和全球范围安全科学的需要,2008年,另一套"监管转基因(GE)植物田间试验标准操作规程(SOPs)和准则"生效。

在印度,监管转基因作物从研发到大规模商业化应用的过程需通过三级法规系统。生物安全委员会机构(IBSC)在研究水平评审操作,遗传操作审查委员会(RCGM)评审所有正在进行的被认为是高风险必须限制田间试验的研究项目。印度官方科学技术部下属的生物安全部门(DBT)对同一机构下的IBSC提供申请认定,同时协助RCGM监管研究和限制田间试验。最后,环境和林业部(MoEF)下属的基因工程评审委员会(GEAC)作为最高主体机构,从环境和商业用途角度,对涉及生物遗传物质改变的研发、生产过程进行审批。在国家层面,SBCC(国家生物技术及协调委员会)、DLC(区级委员会)对以上过程进行检查和监督。

为了解决政策上面临的挑战,政府决定重组监管框架以建立一个科学、严谨、高效、可预知且一致性的监管制度,被称作自主的"国家生物技术管理机构",以提供一个高效的唯一审批通关机制,目前这个决议正在讨论协商中。

3 转Bt茄子案例

3.1 转Bt茄子研发的基本过程

茄子易受病虫害侵袭,其中危害最大的当属FSB(fruit and shoot borer),在茄子的一个生长周期内农民需要喷洒25~80轮杀虫剂。专家估计每年50%~70%的经济损失是由FSB造成的,相当于100亿卢比。当前控制FSB的手段包括喷洒大剂量杀虫剂,结果不仅造成相当剂量的农药残留,还对消费者以及农民的健康构成威胁。转Bt茄子作为一种转基因蔬菜,为防控FSB提供一个防御方法并减少农药施用。大田研究证明,转Bt茄子可以使农民减少70%的杀虫剂施用量,从而达到对FSB的有效控制,而控制所有害虫的农药施用量也减少了42%。田间试验表明,转Bt茄子产量相当于杂交茄子产量的110%,相对于自然授粉品种产量的166%。种植转Bt茄子带来的高产量和高品质使得农民每英亩净收入提高到1 600~1 900卢比,对整个印度增加约合200亿卢比的收入。

在印度,任何转基因作物被释放到环境前,都需要接受严格的生物安全检测,包括环境安全检测,以及由监管部门规定的食品安全检测。本文介绍到目前为止对转Bt茄子进行的所有检测,表明该作物对环境、人类和动物饲养都是安全的。

1)萌发和杂草性试验。萌发测试证明了转Bt茄子和对应非转基因系之间,在种子萌发速率和/或时间方面没有显著性差异。转Bt茄子及其对应非转基因系在生长特性和活力方面也没有差异。这些结果证明转Bt茄子和非转Bt茄子在杂草风险上无实质差异。

2)入侵性试验。本试验测试转Bt茄子同非转Bt茄子入侵性上的差异。该测试若发现植物有任何行为改变,将被认为会对环境造成威胁。这一研究中,在转Bt茄子完全收割后,让种植区闲置3个月后定期灌溉以便那些残留在土壤里的任何种类种子的萌发。

该数据可说明在种植转Bt和非转Bt茄子的土地上,种子的萌发速率以及自然脱落茄子种子的入侵性。任何新萌发、生长的植物都会被检查以确定是否含有转基因成分。结果表明,转Bt茄子种植区没有转基因茄子生长、萌发。该数据说明转Bt茄子没有入侵性和杂草性。

3)两个测试点检测花粉漂移。花粉漂移试验用于研究转Bt茄子花粉的漂移距离,结果发现Bt茄子通过花粉发生漂移的最大距离为20~25 m,而Bt区大多数交叉授粉发生在几米的距离内。

4)对FSB、非靶标昆虫及有益昆虫的影响。在检测的田块里,有无Bt基因的杂交茄子表现出显著的差异性。与其他3个非转基因对照材料相比,转Bt(含*cry1Ac*基因)基因植株上FSB的数量存在显著差异。所有含Bt杂交茄子上FSB的幼虫数量都

显著减少。接虫后对植株的侵害程度测试表明，FSB 对含 Bt 组植株茎秆的损害程度要显著轻于非 Bt 组植株，接虫试验后，转 Bt 茄子和非 Bt 茄子的受损程度显著不同。

为了观察鳞翅目害虫、刺吸式口器害虫和有益昆虫第 2 代的存活情况，观察持续到所有采摘结束。通过对 Bt 组、非 Bt 组以及对照组的比较，未发现发病害虫(蚜虫、小叶蝉、粉虱)和益虫(草蛉、贵妇鸟甲虫、蜘蛛)在数目及种类上存在显著差异。

多地点重复的试验表明，含 Bt 杂交茄子对果梢蛀虫有足够抗性并且有提高产量的潜力。在接虫试验中，含 Bt 杂交茄子比非 Bt 组受损程度显著减轻。含 Bt 杂交茄子对包括有益昆虫在内的非靶标昆虫没有任何影响，因此，含 Bt 杂交茄子可以在茄子的病虫害综合防治策略中发挥积极作用，以实现茄子的可持续种植。

5)对土壤微生物菌落的影响试验。种植转 Bt 茄子，是否会对种植土壤里的微生物菌落、Cry1A(c)蛋白残留，以及土壤无脊椎动物产生影响，本试验针对以上问题进行了多季节、多地点测试。试验明确证明在含 Bt 组和非 Bt 组对比试验里，根际土壤及根际外土壤里的细菌和真菌数量都没有显著差异。关于 Bt 蛋白在土壤残留问题，在作物收获后的土壤样品里已经检测不到 Bt 蛋白。

在定期收集的种植 Bt 和非 Bt 组土壤样品里，细菌和真菌菌群也显示出类似的结果。这些发现证明了 Bt 组土壤样品的 Cry1Ac 蛋白含量低于可检测水平。此外，转基因和非转基因茄子种植区，微生物菌落分布模式相似。

6)实质性等同试验。含 Bt 和非 Bt 组茄子的果实组分分析表明两者具有类似的组分，检测指标包括：蛋白、碳水化合物、油、热量、灰分、氮含量、粗纤维和水分含量等。

同时，对转 Bt 茄子(整合 *Cry1Ac* 基因)和其他 3 个非转基因对照组进行多个组织(组织包括果实、叶片、茎秆和根)化学组分比较，试验数据说明转 Bt 和非 Bt 茄子间无显著差异。

7)Bt 茄子和非 Bt 茄子的化学指纹图谱(生物碱)。转 Bt 茄子和非 Bt 茄子的生物碱含量比较测试在印度海得拉巴的化学科技研究所完成，生物碱的检测步骤如下：先通过氯仿甲醇法萃取生物碱，再用色谱技术定量。结果表明在 TLC 和 HPLC 图谱中，转 Bt 和非转 Bt 的茄子色谱评估无显著差异。

8)大鼠口服急性毒性试验。转 Bt 茄子的口服急性毒性试验由马哈拉施特拉的 INTOX PVT 有限公司完成。将表达 Cry1Ac 蛋白的转 Bt 茄子量按 5 000 mg/kg 的剂量急性喂食斯普拉一道来大鼠(Sprague Dawley rats，SD rats)转基因茄子，并没有引起任何毒性反应。通过口服途径无毒性的蛋白就会被认为通过皮肤或肺途径也无毒性。

9)SD 大鼠(Sprague Dawley rats)口服亚慢性(90 d)试验。本试验也是由马哈拉施特拉的 INTOX PVT 有限公司完成。将转 Bt 表达 Cry1Ac 蛋白的茄子量按超过 1 000 mg/kg 的剂量连续喂食 SD 大鼠90 d后，结果为“观察不到有害效果”(NOAEL)。这项研究证明了表达 Cry1Ac 蛋白的转 Bt 茄子口服喂食试验动物是无毒性的。

10)新西兰白兔喂食亚慢性(90 d)试验。本试验在印度班加罗尔的 Advinus 私人治疗有限公司完成，目的是为了比较含 Cry1Ac 蛋白的转基因 Bt 茄子和对照组对饲喂动物的健康性和安全性影响。基于对饲喂动物健康状况、生长和生理病理参数的分析，表明试验期间喂食含 Bt 茄子和对照组茄子的兔子以上参数测定无显著差异。

11)山羊喂食亚慢性(90 d) 试验。本试验同样由印度班加罗尔的 Advinus 私人治疗有限公司完成。目的与前 2 个试验一样，比较含 Cry1Ac 蛋白的转基因 Bt 茄子和对照组对饲喂动物的健康性和安全性影响。基于对饲喂动物健康状况、生长和生理病理参数的分析，表明试验期间喂食 Bt 茄子和对照组茄子的山羊以上参数测定无显著差异。

12)鱼类饲养试验。本试验由孟买的中央渔业教育研究所完成，试验受体材料为鲤。本试验目的是为了评估喂食转 Bt 茄子对鲤的生长与存活是否存在影响。研究发现，喂食转 Bt 茄子的鲤与喂食非转基因茄子的鲤的生长模式类似。这些结论是通过对 2 组鲤的生长反应和组织病理学变化进行比较得出的。

13)对肉鸡健康的影响试验。喂食鸡的试验由印度 Izatnagar 禽类研究中心完成。目的是为了评估表达含 Cry1Ac 蛋白的转 Bt 基因茄子对肉鸡的生长和营养吸收有无影响。结果显示，2 组试验肉鸡的体质量增长量、饲料摄取量和饲料转化率均无显著差异，血液的生化成分也没有显著差异。结论是，转 Bt 茄子作为肉鸡的饲料和非转基因茄子同样安全。

14)喂食哺乳期杂种奶牛试验。喂食奶牛的试验由位于潘特纳加的GBPUAT完成。对喂食转Bt基因和非转基因茄子的哺乳期奶牛进行以下营养价值指标的考察:饲料摄取量、牛奶产量和牛奶组分。牛奶组分测定以确定奶牛的牛奶和血液中是否含有Bt蛋白的存在。结论是2组营养价值指标相似,转基因茄子没有对哺乳期奶牛产生任何不利影响。

15)煮熟后蛋白质评估。在印度,茄子通过多种烹饪方式煮熟后食用。煮熟后蛋白质评估由Mahyco公司实施,他们采用大部分烹饪方式来评估转Bt基因茄子中是否含有Cry1Ac蛋白。采用未烹煮的转Bt茄子作为ELISA的阳性对照,非转基因Bt茄子作为ELISA的阴性对照。茄子收获后放冰上带去实验室进行多种方式的烹煮,提取烹煮后茄子总蛋白并用ELISA检测Bt蛋白的含量。无论使用何种烹饪方式(烤、浅煎、油炸或清蒸),都无法在第一取样时间(烤5 min,其他均1 min)的样品中检测到Bt蛋白。这一结果说明通过烹煮后转基因Bt茄子里的Cry1Ac蛋白会很快降解。

16)社会经济学研究。许多学术团体如Chong、Krishna和Qaim已对种植转Bt基因茄子开展了社会经济学的研究。他们的工作表明农民乐于接受这种新技术,杀虫剂用量的减少和茄子产量的提高可增加农民的收益。

3.2 现 状

印度环境和森林部已于2010年暂停了该项目,目前转Bt茄子还在等待最后的决定。与此同时,孟加拉已经于2013年批准了该品种的转基因茄子在孟加拉的商业化种植,农民已经将产品投放市场,并且看到期望中的抗虫效果。他们已打算在下一季扩大该品种的种植面积。

表1 田间试验种植转基因作物的公共研究所列表

Table 1 A list of fields trials of GM Crops being conducted by public research institutions

编号 No.	作物名称 Crops	年份 Year	研究所 Institute	性状 Traits
1	茄子 Brinjal	2006	IARI,New Delhi	抗虫 Insect resistance
2	蓖麻 Castor	2006	Directorate of Oilseeds Research, Hyderabad	抗虫 Insect resistance
3	落花生 Groundnut	2006	ICRISAT, Hyderabad	抗病毒 Virus resistance
4	马铃薯 Potato	2006	Central Potato Research Institute, Shimala	抗真菌 Fungal resistance
5	水稻 Rice	2006	IARI,New Delhi	抗虫 Insect resistance
6	水稻 Rice	2006	TNAU, Coimbatore	抗病 Disease resistance
7	西红柿 Tomato	2006	IARI,New Delhi	抗病毒 Virus resistance
8	茄子 Brinjal	2007	UAS,Bangalore	抗虫 Insect resistance
9	茄子 Brinjal	2007	TNAU, Coimbatore	抗虫 Insect resistance
10	马铃薯 Potato	2009	Central Potato Research Institute, Shimala	块茎增甜 Tuber sweetening
11	鹰嘴豆 Chickpea	2009	ICRISAT, Hyderabad	抗非生物胁迫 Abiotic stress tolerance
12	高粱 Sorghum	2009	National Research Centre for Sorghum, Hyderabad	抗虫 Insect resistance
13	西瓜 Watermelon	2010	Indian Institute of Horticultural Research	抗病毒 Virus resistance
14	西红柿 Tomato	2010	Indian Institute of Horticultural Research	抗病毒 Virus resistance
15	西红柿 Tomato	2010	IIVR, Varanasi	抗虫 Insect resistance
16	西红柿 Tomato	2010	NRCPB, New Delhi	果实催熟 Fruit ripening
17	番木瓜 Papaya	2010	Indian Institute of Horticultural Research	抗病毒 Virus resistance
18	甘蔗 Sugarcane	2010	Sugarcane Breeding Institute	抗虫 Insect resistance
19	高粱 Sorghum	2010	Central Research Institute for Dryland Agriculture	抗非生物胁迫 Abiotic stress tolerance
20	落花生 Groundnut	2010	University of Agricultural Sciences, Bangalore	抗非生物胁迫 Abiotic stress tolerance
21	芥末 Mustard	2010	NRCPB, New Delhi	抗非生物胁迫 Abiotic stress tolerance
22	芥末 Mustard	2010	University of Delhi South Campus, Delhi	杂种优势 Heterosis
23	落花生 Groundnut	2011	ICRISAT, Hyderabad	抗真菌 Fungal resistance
24	橡胶 Rubber	2011	Rubber Research Institute, Kottayam	抗非生物胁迫 Abiotic stress tolerance

4 展 望

对转基因作物的审批仅仅限于有限的田间试验,仅表1中所列出的公共机构才能开展多地点的田间试验。此外,许多农艺性状的改良正在努力研发,这样我们需要推进审查进度,尽快将这些产品释放到农民的田里。

转基因争论是数十年来农业领域最为激烈的话

题。然而,转基因食品被数百万的消费者食用已超过17年,目前尚未有任何对人类健康有负面影响的报道。在发达和发展中国家,规模不等的大小农场都意识到了种植转基因作物带来的收益。转基因作物监管系统是完善的,我们需要提高科学界以外的普通民众对转基因作物的信心,一旦通过规定程序的审查确定是安全的,转基因作物就可以投放生产。转基因作物可以在农业生产力的可持续提高中发挥重要作用。

GM crops in India

Usha Barwale Zehr

Maharashtra Hybrid Seeds Company Ltd, Dawalwadi, Maharashtra, India

Abstract Regulatory approval for commercial cultivation of biotech cotton in India was granted in 2002. The 2014 cotton growing season saw that approx. 95% of acreage planted to cotton was either a single gene or two gene product of cotton. Adoption of Bollgard and BollgardII cotton in India lead to a sea change in the cotton sector, leading to benefits at the farm, farmer and national level. While this has been a big success, India has not commercialized any crop since the commercial release of the GM cotton and this paper will discuss some of the current challenges, the regulatory system and the readiness of the researchers to bring out new products, subject to government approvals.

Key words GM crops; regulatory approvals for GM crops; India

翻　译: 吴　昊　　**校　正:** 吴昌银　华中农业大学作物遗传改良国家重点实验室

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Abstract Regulatory approval for commercial cultivation of biotech cotton in India was granted in 2002. The 2014 cotton growing season saw that approx. 95% of acreage planted to cotton was either a single gene or two gene product of cotton. Adoption of Bollgard and BollgardII cotton in India lead to a sea change in the cotton sector, leading to benefits at the farm, farmer and national level. While this has been a big success, India has not commercialized any crop since the commercial release of the GM cotton and this paper will discuss some of the current challenges, the regulatory system and the readiness of the researchers to bring out new products, subject to government approvals.
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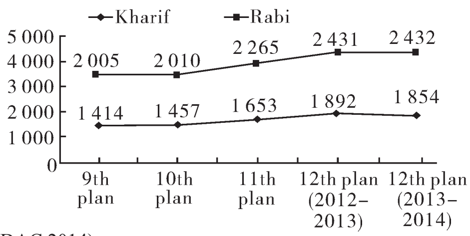
1 Introduction

India has made tremendous progress in terms of food security and self-sufficiency since the green revolution. The gains seen in the green revolution which led to quantum jumps are now plateauing. The most recent report of the 2012-2013 and 2013-2014 seasons shows that the productivity is levelling off (Figure 1). While the rate of growth in agriculture is slowing down as seen by these numbers, the demand for food remains. In addition,With rising incomes, the types of foods needed by the population demand a diverse agriculture system with enhanced focus on vegetables, legumes and fruits. With the growing Indian population, impact of climate change and dwindling natural resources, we need to continue to improve productivity of diverse group of crops to ensure food security, beyond rice and wheat in years to come.

Technology continues to advance at a fast pace and the use of these tools in agriculture is very much required to see that the benefits of these flow to farmers and ultimately to the consumers. The losses incurred today in various crops due to insects, diseases and various abiotic stresses again highlight the need for applying advances in science and technologies to lower these losses. Insect tolerant GM cotton crop has been hugely successful and we need to see the other crops and traits which are under development also be planted on farmers fields. Molecular breeding is being used more extensively in few commercial crops and that too will lead to superior germplasm and products. India has a strong regulatory system in place and the list of products under various stages of development include multiple crops like okra, tomato, cabbage, cauliflower, maize, rice, wheat, chickpea, rubber and many more with traits which address insect tolerance, herbicide tolerance, fertilizer use efficiency, salt tolerance, drought tolerance, seed production technologies, disease tolerance and others.



(DAC 2014)

Fig. 1 Productivity of food grains in kg/ha (Kharif & Rabi) over IX, X, XI Plans and 2012-2013 & 2013-2014

2 The first biotech crops

Insect-resistant Bt cotton varieties were successfully approved for commercial cultivation in 2002. The two gene combination was approved in 2006. Several other products were next in the pipeline such as mustard, potato, brinjal, okra, rice. However, the moratorium on Bt brinjal by Ministry of Environment and Forest in 2010 meant a considerable detour from an objective, science-based, rigorous regulatory approval process to a more subjective, non-science driven political decision making process.

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3 Biotech regulation in India

Indian Acts, rules and regulations as well as procedures for handling of genetically modified organisms (GMOs) and rDNA products have been formulated under the Environment (Protection) Act (EPA) 1986 and Rules 1989. The rules in general cover manufacture, use/import/export and storage of hazardous micro-organisms, genetically engineered organisms or cells and came into force from 1993. A set of rDNA guidelines were issued in 1990 covering genetically engineered organisms, genetic transformation of plants and animals, mechanism of implementation of biosafety guidelines and containment facilities under three risk groups. The guidelines have been revised matching with the needs of scientific knowhow in 1994 as "Revised Guidelines for Safety in Biotechnology". During 1998, to provide special review for genetically engineered plants, "Revised Guidelines for Research in Transgenic Plants and Guidelines for Toxicity and Allergenicity for Evaluation of Transgenic Seeds, Plants and Plant Parts" had come into force. In response to changing needs of transgenic technology and safety science globally, in 2008 another set of "Guidelines and standards for operating procedures (SOPs) for confined field trials of regulated genetically engineered (GE) plants".

In India, regulatory set up oversees the development of GM (genetically modified) organisms including cropsfrom the research stage to large-scale commercial use through a three-tier system. The *Institutional Biosafety Committee* (IBSC) operates at research level approvals and the *Review Committee on Genetic Manipulation* (RCGM) reviews all approved ongoing research projects which are considered to be in the high-risk category and controlled field experiments. The Department of Biotechnology (DBT) under the Ministry of Science and Technology, Govt. of India provides recognition to IBSC which is in the institution making the applications and also services RCGM for regulating research and limited field experiments. Finally, *Genetic Engineering Approval Committee* (GEAC) functions as an apex body under Ministry of Environment and Forests (MoEF) and is responsible for the approval of activities involving large-scale use of hazardous microorganisms as well as recombinant products in research and industrial production from an environment angle or commercial use. At State Level, SBCC (state biotechnology and co-ordination committee), DLC (district-level committee) inspect, supervise and involve monitoring with the help of scientists from state and central government institutions.

To address the policy challenges the government had decided to restructure the regulatory framework so as to establish a scientific, rigorous, efficient, predictable and consistent regulatory regime articulated as autonomous'National Biotechnology Regulatory Authority' to provide effective single window clearance mechanism which is still under discussion.

4 Bt brinjal as a case study

4.1 Rationale for the development of Bt brinjal

The brinjal fruit and shoot borer (FSB) is the most damaging pest on brinjal crops, and farmers need to spray 25-80 rounds of pesticides during each growing season. Experts estimate that the financial loss to the country because of the 50%-70% of damage caused by the FSB is equivalent to Rs 1 000 crore per annum. As present control methods for FSB involve heavy pesticide sprays on the crop, brinjal produce potentially contains significant amounts of pesticide residues, posing health concerns for consumers as well as farm workers. Bt brinjal, a genetically modified (GM) or biotech crop, provides an alternative method for FSB control and pesticide application reduction. Field studies with Bt brinjal have demonstrated that farmers can use 70% less insecticide for FSB control and, as a result, 42% less pesticide overall for control of all insect pests. Field studies have shown that this results in an average 116% increase in marketable fruits over hybrids and 166% increase over open-pollinated varieties of brinjal. The higher yield and better quality would result in higher net income for brinjal farmers to the tune of Rs 16 000-19 000 per acre, which works out to Rs 2 000 crore to farmers over India as a whole.

Before any biotech food crop can be released in the environment in India, it has to undergo stringent biosafety tests, including environmental safety testing as well as food safety testing mandated by the regulatory authorities. This article describes tests conducted on Bt brinjal so far, which indicate that this crop is safe to the environment and safe for human and animal consumption.

4.2 Germination and weediness studies

Germination tests demonstrated that there is no significant difference in the rate and/or time taken for germination between Bt brinjal and its non-Bt counterpart. Bt brinjal and its non-Bt counterpart did not differ in growth characteristics or vigour. These results demonstrated that there is no substantial difference between transgenic Bt and non-Bt brinjal with regard to their potential for weediness.

4.3 Aggressiveness studies

A study was conducted to monitor the aggressiveness of Bt brinjal as compared to its non-Bt counterparts. This assesses any change in plant behaviour which could lead to the crop posing a risk in the environment. In the study, after complete harvesting of the Bt brinjal crop, the area under planting was left undisturbed for three months and irrigated on a regular basis to allow for germination of any seeds that might have remained in the ground after harvesting the main crop.

The data provides information on germination rates and aggressiveness of naturally shed brinjal seeds under field conditions in the plots where Bt and non-Bt plants have been grown. Any plants emerging are checked to determine the presence of the biotech trait. In the study conducted on Bt brinjal there were no brinjal plants observed to grow or germinate in the plot over the period of the study. The data suggest that there is no aggressiveness or weediness demonstrated by Bt brinjal plants.

4.4 Pollen flow studies-2 Locations

Pollen flow studies on Bt Brinjal were conducted by to determine the distance traversed by pollen from Bt brinjal plants, which was found to be 20-25 m. The majority of cross-pollinations occurred within a few metres of the Bt plot.

4.5 Effect on fruit and shoot borer, non-target insects and beneficial insects

In controlled field trials, significant differences were detected between hybrids based on presence or absence of Bt gene. For FSB counts, significant differences were detected between Bt hybrids (containing *cry1Ac* gene) and all three non-Bt checks. All Bt hybrids were significantly lower in number of FSB larvae. Differences were also measured between the Bt hybrid and non-Bt check hybrids for shoot damage to plants from FSB infestation. Percent damage to shoots was significantly lower for the Bt group as compared to non-Bt hybrids. The degree of such differences in FSB feeding damage between Bt hybrids and non-Bt hybrids was significant.

Observations were taken till completion of all pickings, for the presence of secondary lepidopteran pests, sucking pests and beneficial insects. No significant differences were noted between Bt hybrids, the non-Bt counterparts and checks in terms of incidence of sucking pests (aphids, jassids, whitefly) and beneficial insects (chrysopa, lady-bird beetle, spiders).

Results of these multi-location replicated research trials indicate that the Bt brinjal hybrids provide adequate level of tolerance to BFSB and show good yield potential, BFSB efficacy and marketable yield. Bt brinjal hybrids showed a significantly lower damage resulting from BFSB feeding in comparison to non-Bt brinjal. Bt brinjal hybrids did not have any effects on non-target insects, including beneficial insects; and therefore can play a positive role within integrated pest management strategies for sustainable brinjal cultivation.

4.6 Effect on soil micro-microflora studies

The effect of growing Bt brinjal in open field on soil microflora, residue of Cry1A(c) protein and soil invertebrates was studied over a number of growing seasons and locations. It was clearly demonstrated that there were no differences between Bt and non-Bt plots vis-à-vis soil bacteria and fungal count both at the rhizosphere and the soil beyond the rhizosphere. Regarding residual Bt protein in the soil, after harvest of the crop it was found to be non-detectable in any of the soil samples tested.

Similar results in terms of bacterial and fungal populations were obtained from soil samples collected periodically from Bt and non-Bt brinjal plots. These findings demonstrate that Cry1Ac levels as determined through bioassays in soil samples was below detectable levels in soil samples collected from Bt brinjal plots. Further, microbial populations from Bt and non-Bt plots showed similar patterns.

4.7 Substantial equivalence studies

Compositional analysis of Bt and non-Bt brinjal fruit showed similarity in composition when major components like protein, carbohydrate, oil, calories, ash, nitrogen, crude fibers and moisture contents were analyzed.

A comparative study for the chemical composition of the tissues of brinjal plants was made using Bt brinjal (incorporated with *cry1Ac* gene) entries and three non-Bt controls. The chemical composition was determined in the fruit, leaf, stem and root tissues of the brinjal plant. The data obtained in this study indicated that there were no appreciable differences between Bt brinjal and non-Bt brinjal groups in the chemical constituents of moisture, proteins, oil, ash, carbohydrates, calories for fruit tissue and nitrogen, ash and crude fiber contents in leaf, stem and root tissues.

4.8 Chemical fingerprinting of Bt and non-Bt brinjal (alkaloids)

Estimation of the alkaloid content in Bt brinjal in comparison with its non-Bt counterpart was done at Indian Institute of Chemical Technology, Hyderabad.

Assessment of the presence of alkaloids in Bt and non-Bt brinjal fruit was done by chloroform and methanol extraction method using chromatographic techniques. There were no significant differences in TLC and HPLC profiles of Bt and non-Bt brinjal fruit in chromatographic analysis for alkaloid estimation.

4.9 Acute oral toxicity studies in rats

Acute oral toxicity study of transgenic Bt brinjal was conducted at INTOX PVT. LTD. , Pune, Maharashtra, India to assess the safety of Bt brinjal. Acute oral administration of transgenic Bt brinjal expressing Cry1Ac protein to Sprague Dawley rats at the limit dose of 5 000 mg/kg did not cause any toxicity. Proteins that are non-toxic by the oral route are not expected to be toxic by the dermal or pulmonary route.

4.10 Sub-chronic (90 days) oral toxicity study in Sprague Dawley rats

Subchronic oral (90 days) toxicity study of transgenic Bt brinjal in Sprague Dawley Rat was conducted at INTOX PVT. LTD. , Pune, Maharashtra, India. Based on the findings of this study, the no-observed-adverse-effect-level (NOAEL) of transgenic Bt brinjal expressing Cry1Ac protein in Sprague Dawley rat, following oral administration for 90 days was found to be more than 1 000 mg/kg body weight. This study demonstrates that Bt brinjal expressing Cry1Ac protein is non-toxic to the study animal by oral route.

4.11 Sub-chronic (90 days) feeding studies using New Zealand white rabbit

Subchronic (90 days) rabbit feeding studies were conducted on New Zealand White rabbits at Advinus Therapeutics Private Ltd., Bangalore, India. The objective of this study was to compare the wholesomeness and safety of transgenic Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. As per the findings of this study, it was concluded based on the health, growth and physio-pathological parameters analysed during the experiment that there were no significant differences between the groups fed with transgenic Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

4.12 Sub-chronic (90 days) feeding studies in goats

Subchronic (90 days) goat feeding studies were conducted at Advinus Therapeutics Private Ltd. , Bangalore, India. The objective of this study was to compare the wholesomeness and safety of transgenic Bt brinjal containing *cry1Ac* gene with control non-Bt brinjal. As per the results of this study, it was concluded based on the health, growth and physio-pathological parameters analysed during the experiment that there were no significant differences between the groups fed with transgenic Bt brinjal containing *cry1Ac* gene and control non-Bt brinjal fruit.

4.13 Feeding studies on fish

A fish feeding study was conducted at Central Institute of Fisheries Education, Mumbai, India using the common carp, *Cyprinus carpio*. The objective of this study was to evaluate Bt brinjal as a feed ingredient for common carp and to study the comparative growth and survival of fish fed with Bt brinjal. The study found that fish fed with Bt brinjal showed similar growth patterns to those fed with non-transgenic brinjal. Bt brinjal, non-Bt brinjal-fed groups were found to be statistically similar in terms of fish growth responses, and histopathological alterations.

4.14 Effect on health of broiler chickens

A chicken feeding study was conducted at Central Avian Research Institute, Izatnagar, India. The objective of this study was to assess the impact of transgenic Bt brinjal expressing *cry1Ac* gene on chickens, in terms of growth performance and nutrient utilization. Results of the present study showed that body weight gain, feed intake and feed conversion ratio did not differ among Bt and non-Bt treatments. Several blood biochemical constituents did not differ statistically due to dietary treatments including Bt and non-Bt brinjal incorporated diets. This study found Bt brinjal to be as safe as non-transgenic brinjal in terms of responses of chickens fed with diet incorporating the two types of brinjal.

4.15 Feeding studies in lactating crossbred dairy cows

Cow feeding studies were conducted at GBPUAT, Pantnagar, to assess the nutritional value of transgenic Bt brinjal fruit in comparison with non-Bt brinjal fruit in lactating crossbred dairy cows in terms of feed intake, milk production and milk composition and to determine if the Bt protein was detectable in milk and blood of lactating crossbred dairy cows fed on ration containing transgenic brinjal fruits. From the study it was concluded that the nutrional value of both transgenic and non-transgenic brinjal fruits were similar in terms of feed intake, milk yield and milk constituents without any adverse effects on the health of lactating crossbred dairy cows.

4.16 Food cooking and protein estimation in cooked fruits

Cooked brinjal fruits are consumed in various forms in India. Cooking studies carried out at Mahyco included most of the forms in which brinjal fruits are consumed. Tender brinjal fruits expressing the *cry1Ac* gene (henceforth referred to as Bt brinjal) were used in these studies to determine whether the Bt protein was present in the cooked fruits. Uncooked Bt fruits and non-Bt fruits were used as positive and negative controls respectively, for ELISA. The fruits were harvested and brought to the lab on ice and used in different cooking experiments. The protein extracts from these samples were used in ELISA for the detection of Bt protein. The Bt protein was undetectable in the cooked fruits at the first sampling time-point irrespective of the cooking method used (roasted, shallow-fried 1, deep-fried or steamed). The first sampling time-point was 5 min for roasted fruit and 1 min for the other forms of cooking. This study indicates that the Cry1Ac protein in Bt brinjal fruits is rapidly degraded upon cooking.

4.17 Socioeconomic studies

A number of socioeconomic studies have been carried out by academic groups such as those by Chong, and Krishna and Qaim, indicate a receptiveness of farmers to the technology, and the potential of Bt brinjal to increase farmers' welfare through insecticide reductions, and an increase in marketable yields of brinjal.

4.18 Current status

A moratorium was imposed in 2010 by the then Minister of Environment and Forest and still awaiting a final decision. In the meantime, Bangladesh has commercially released this event in Bangladeshi varieties for cultivation in 2013 and the farmers took the produce to market and were able to see the expected control against the target pest. They are planting their second season of the crop on a larger acreage.

5 Looking ahead

Transgenic crops approved for conducting contained/confined limited field trials, including multilocation field trials only from the public institutions are listed in Table 1. In addition, industry is also working on numerous traits. The pipeline is strong and we need to move forward in the regulatory process to bring these products to the farmers fields.

The GM debate has been the most divisive debate we have seen in Agriculture for decades. However, no negative health effects due to GM foods have been documented in humans after being consumed for 17+ years by millions of people. Also the benefits of the GM crops have been realized by small and large farms, in developed and developing countries. The regulatory systems are robust. The need is to create higher level of confidence amongst the non-scientific community and general public so as to bring the pipeline products to market once they have cleared all biosafety requirements. Enhancing productivity in a sustainable manner is the need and GM crops can play an important role.

Table 1 A list of field trials of GM crops being conducted by public research institutions

S. No	Crops	Year	Institute	Traits
1	Brinjal	2006	IARI, New Delhi	Insect resistance
2	Castor	2006	Directorate of Oilseeds Research, Hyderabad	Insect resistance
3	Groundnut	2006	ICRISAT, Hyderabad	Virus resistance
4	Potato	2006	Central Potato Research Institute, Shimla	Fungal resistance
5	Rice	2006	IARI, New Delhi	Insect resistance
6	Rice	2006	TNAU, Coimbatore	Disease resistance
7	Tomato	2006	IARI, New Delhi	Virus resistance
8	Brinjal	2007	UAS, Bangalore	Insect resistance
9	Brinjal	2007	TNAU, Coimbatore	Insect resistance
10	Potato	2009	Central Potato Research Institure, Shimla	Tuber sweetening
11	Chickpea	2009	ICRISAT, Hyderabad	Abiotic stress tolerance
12	Sorghum	2009	National Research Centre for Sorghum, Hyderabad	Insect resistance
13	Watermelon	2010	Indian Institute of Horticultural Research	Virus resistance
14	Tomato	2010	Indian Institute of Horticultural Research	Virus resistance
15	Tomato	2010	IIVR, Varanasi	Insect resistance
16	Tomato	2010	NRCPB, New Delhi	Fruit ripening
17	Papaya	2010	Indian Institure of Horticulture Research	Virus resistance
18	Sugarcane	2010	Sugarcane Breeding	InstituteInsect resistance
19	Sorghum	2010	Central Research Institute for Dryland Agriculture	Abiotic stress tolerance
20	Groundnut	2010	University of Agricultural Sciences, Bangalore	Abiotic stress tolerance
21	Mustard	2010	NRCPB, New Delhi	Abiotic stress tolerance
22	Mustard	2010	University of Delhi South Campus, Delhi	Heterosis
23	Groundnut	2011	ICRISAT, Hyderabad	Fungal resistance
24	Rubber	2011	Rubber Research Institute, Kottayam	Abiotic stress tolerance