To July 16, 2006

Shri Bir Singh Parsheera
Chairperson, Genetic Engineering Approval Committee [GEAC]
Ministry of Environment & Forests
Paryavaran Bhawan, CGO Complex
Lodhi Road, New Delhi

Dear Shri Parsheera

Sub: Feedback on Bt Brinjal – biosafety & beyond

Sir, by now you would have received the initial feedback provided to the Minister for Environment & Forests with a copy marked to you, on June 15th 2006, on the issue of Bt Brinjal and its very need. We are annexing that response to this letter for your ready reference [Annexure 1].

We would like to first record our serious objection to multi-locational limited field trials being conducted in various locations in the open environment, in farmers' fields ***without*** biosafety being cleared, without adequate monitoring and containment capabilities and very often, in violation of farmers' rights. No liability has been fixed for biosafety violations pointed out earlier, giving a clear message that biosafety is not the regulators' primary concern at all, though the GEAC has been created, constituted and allowed to function expressly for that.

The following is the collective feedback from the Coalition for GM-Free India which touches upon the earlier-made points even as it includes newer feedback on the biosafety testing protocols that have been adopted in the case of Bt Brinjal and the results of the tests.

1. We repeat that **there is no need for Bt Brinjal** to be introduced. This is not something that farmers have demanded and almost all major farmers' organizations of the country have already rejected the proposal of entry of Bt Brinjal even if it is in the form of field trials and seed production, whether in the public sector or in the private sector. There is no crisis in the production of brinjal and it is absolutely false that the company's data claims that there are upto 80 sprays of pesticides on brinjal crop. For the consumers, there is absolutely no benefit with Bt Brinjal but only a set of problems and dangers presented. All major consumer organizations of the country have already rejected the idea of Bt Brinjal. If despite the lack of need and demand, Bt Brinjal is permitted for field trials and seed production, what guarantee is GEAC is giving to us that this is indeed safe? What liability-fixing mechanisms exist to hold each individual member of GEAC accountable for these kinds of decisions taken?

2. There are other issues on which Brinjal farmers need intervention & support. Are the GEAC and concerned government ministries and departments giving **any guarantee to farmers that they will procure the crop and stabilize prices** with Minimum Support Price and guaranteed procurement to ensure a fair price to farmers? If not, what real benefits will accrue to farmers?

3. **What guarantee are GEAC and the individual members who represent ministries that are mandated to protect consumer interests** giving that consumer rights and choices will be upheld even after the entry of Bt Brinjal, if approved? Will labelling work for distinguishing between Bt and non-Bt Brinjal in our markets and *haats*? What choices are being left to consumers of the country who want to remain GM-free in their consumption and how will their fundamental right to safe food be upheld?

4. If Bt Brinjal is for reducing pesticide usage, then it has to be noted that Bt Brinjal has been compared with only conventionally-grown brinjal. This completely ignores the rich experience that exists within the Indian Council of Agricultural Research [ICAR] on Integrated Pest Management on brinjal, that too with non-chemical approaches. It also ignores the fact that there is vast experience with NPM and organic approaches which farmers have been successfully using for years now on a large scale. Does GEAC have data on such experience and **does the Committee know how Bt Brinjal compares with such IPM/NPM/Organic experience?**

5. Brinjal has **great socio-cultural significance** in the country. There are communities where a wedding feast is not complete without a special brinjal preparation. Does the GEAC or the company have adequate information/data on such aspects and what the impact of Bt Brinjal would be on such socio-cultural dimensions?

6. **Brinjal is also used in Ayurveda for its medicinal properties** [we are attaching a note from a Ayurveda Vaidya on this issue as Annexure 2]. Does GEAC or the company have data on this and about what Bt Brinjal's impacts on the efficacy of such medicines would be?

7. Coming to the "biosafety tests" that have been conducted on Bt Brinjal – it is repeated again through this letter that **no independent studies have been taken up** to test the biosafety of Bt Brinjal. The entire regulatory mechanism is relying on the developers of the product to come back to the regulators and actually report that something is indeed wrong with the process or product! This is of course impossible to happen and the past history with biotech corporations shows that companies like Monsanto have willfully suppressed information evolved through own investigations on harmful effects of GM crops. There is completely unacceptable conflict of interest in this matter and GEAC should therefore **not take any decisions** based on this set of studies and findings.

8. Initially, GEAC put up only the presentation made by M/s Mahyco and only later, after much protest from civil society groups was other information put up. However, we find that even now, while protocols that have been approved by DBT have been put up, no numbers in terms of findings are **not** available for the majority of tests conducted. Without looking at numbers, no intelligent feedback is possible.

   Most of what is given below looks at shortcomings in the protocol and where possible, compares the findings with findings from other studies elsewhere. We would like to record our protest strongly here that opening the records and documents for only those people who can come all the way to Delhi for looking at the full biosafety data, precludes others from giving their expert opinions and feedback - it is only making this entire process of obtaining feedback a farcical one.

9. It is obvious from all the tests that they were done in great haste, to appease the regulatory requirements rather than to genuinely test for any potential adverse impacts, especially **in the medium and long term**. Very important tests including the effect of Bt toxin combined with pesticides [combination effect] was not taken up anywhere whereas this would be the reality of cultivation practices even with GM technology! In addition, there is of course the whole area of "unintended consequences" where the regulators and others do not even know the right questions to ask!

10. Data from elsewhere shows that there are serious health hazards connected with the genes used in making Bt Brinjal. The Cry toxins from Bt are known to be allergens and

immunogens. The antibiotic-resistant marker genes [*aad* and nptII genes] bring their own serious concerns with regard to the safety of the product. The nptII gene confers resistance to antibiotics like kanamycin and neomycin. The *aad* gene confers resistance to streptomycin and spectinomycin. Further, the *aad* gene is under the control of a bacterial promoter. Both genes and DNA can theoretically get transferred into bacteria and cause antibiotic resistance. In a country which depends on antibiotics like streptomycin in its healthcare, this could be a dangerous development.

11. Similarly, use of the CaMV 35 S [cauliflower mosaic virus] promoter, used in creating Bt Brinjal is a matter of concern. Published research shows that the 35S promoter can initiate transcriptional activity in human cells, despite the promoter being a plant-specific one. The cauliflower mosaic virus (CaMV) has similarities with the human hepatitis B virus. As all genomes of living species contain dormant viruses, there is a potential for the CaMV promoter to reactivate them raising concerns related to cancers.

12. **Pollen Flow studies**: The pollen flow studies with regard to Bt Brinjal were done in two locations during 2002.

    a. It is not clear how the pollen flow studies have been taken up in the same year that the backcrossing programme began!!
    b. The one year study taken up is grossly inadequate to understand the potential contamination of and transfer to other species from Bt Brinjal and **such studies require at least 5 years in different locations to understand the potential impacts.**
    c. The Mahyco presentation itself talks about brinjal being cross-pollinated to an extent as high as 48% and 'is often classified as a cross-pollinated crop'. Other references are available which record a similar level of outcrossing. Outcrossing primarily takes place with the help of insects.
    d. The pollen flow studies done in the case of Bt Brinjal do **not** assess the distance traveled by the transgene though the objective states so. The counting of spiny seedlings from the non-spiny Pusa Kranti brinjal variety's progeny also does **not** indicate outcrossing percentage of the transgene. It only measures the outcrossing of other traits and not the transgenic trait, which is of utmost concern.
    e. Pollen travel distance was concluded as 20 meters and outcrossing percentrage as 1.5% to 2.7% based on this protocol with serious shortcomings. These results are highly undependable, both because the protocol is faulty and because the results are inconsistent with known information on such outcrossing. This outcrossing will obviously be a combined result of several factors, including the fact that insect load and activity itself might be low in a given situation [like the company's campus]. This insect activity could also vary across kharif and rabi seasons. Therefore, what comes out of the limited testing by Mahyco in its campuses cannot obviously be generalized to all brinjal-growing situations in the country.
    f. In the protocol adopted here [concentric rings of Pusa Kranthi non-spiny hybrid around the Bt Brinjal plot], the movement of pollen gets effected drastically by the pollen load / density, micro-climate, physical hindrances etc. created by the crop (Pusa Kranti) taken around in concentric rings. As we all know, this is one of the factors always considered in modifying / reducing the isolation distances in seed production programs. This pollen load and density will also be affected by the size of the Bt Brinjal block in the middle. It is not clear from the data provided by the company how big the transgenic brinjal plot was in the middle.

g. The study only looks at the potential transfer from one cultivated variety to another. It does not look at a whole set of issues related to potential transfer to wild and other related varieties and the subsequent impact on the eco-systems that are present for each of these varieties.
h. We also note that there is no data or study of pollen viability which is also an important factor to consider, talking about outcrossing and insect pollination.
i. Does GEAC or the company have data on all the related species to brinjal, wild and otherwise and where these exist? Do they have data on the eco-systems of such areas? Have they done any tests to understand the potential impacts of Bt Brinjal on such varieties and their eco-systems?
j. The pollen flow studies should actively look at exceptional pollination events, since India is a centre of origin for brinjal.
k. There is no data on other methods of propagation including seed spillage etc. The weediness tests [and test for volunteers] are completely inadequate and even one volunteer is a potential source of cross-pollination later on.
l. The company-adopted protocol is obviously faulty, inapplicable to real growing conditions and has not obviously tested for transfer to wild varieties and the possibility of Bt Brinjal conferring an advantage to them.

13. **Agronomic trials**: It is not clear how the agronomic trials were conducted in Kharif 2004 and Kharif 2005 by the company. For each hybrid, multi-locational trials were conducted for only one year, in two locations only, based on which fruit damage and average fruit damage is being reported to be dramatically different between the Bt and non-Bt brinjal plots. The agronomic trials conducted by ICAR was for two years for the first set of five hybrids and for one year for another 3 Bt Brinjal hybrids. However, data from the first year of trials is not available in public domain and no intelligent feedback can be provided without information on the protocols used and the complete set of data generated.

    a. What was the protocol adopted for the company's trials? Did it compare the Bt Brinjal with other alternatives like IPM, NPM, organic etc.?
    b. Who oversaw the data generated by the company, for each location and what were their monitoring findings?
    c. The agronomic performance overseen through the ICAR trials has misleading data and conclusions. This is unreliable since the data has not been statistically analysed. Even the data presented by Mahyco from its own limited field trials in farmers' fields shows a 7-fold variability in yields per hectare, across hybrids and a 2.5-fold variability within a hybrid. This skews the averages quite a bit.
    d. These trials did not compare Bt Brinjal with other alternatives including non-chemical IPM, IPM, NPM and organic.
    e. There were at least three centres [of ICAR trials] which did not report back their results. The reasons have to be looked into.
    f. There is no data available on the economics of Bt Brinjal cultivation, though marketable yields are being reported.
    g. There is no data available on the reduction in pesticide use, the main grounds on which Bt Brinjal is being brought in. Informal reports from at least one Centre of the ICAR-supervised trials indicate that pesticide use on the trial plot was really high.
    h. All ICAR trials are paid up trials, no independent assessment was made. There is a serious and objectionable conflict of interest in this.
    i. Our own investigations reveal that many of the scientists involved in such trials are also not adequately trained on biosafety issues and testing protocols.

14. **Soil impact studies**: There are several serious shortcomings with the protocol adopted for the soil analyses related to Bt Brinjal cultivation.

- The soil impact studies have not been conducted to capture cumulative effects over several years of Bt Brinjal cultivation and have only analysed impacts for one season each
- To estimate the impact of Bt toxin on soil microorganisms involves isolation and enumeration of micro-biota and study of biochemical characteristics for utilization of certain chemicals (or) compounds (or) production of metabolites by their physiological characteristics. Enzyme studies and finally the molecular behaviour of the genes responsible for particular characteristics have to be understood for changes.
- The physiological and molecular aspects were not studied in different treatments in these soil analysis tests. Similarly accumulation of toxin through the leaf litter was not taken up, as the soil samples were collected on pre-harvest days. The toxin persistence in the soil seems unestimated.
- The study results did not reveal the following: what are the lethal levels of toxins to kill the test invertebrates? What are the actual toxin levels in the soil in the pre-harvest and post-harvest seasons of Bt and non-Bt crops? What are the changes in enzymatic and physiological behaviour in soil biota? What are the genetic modifications that took place affecting the functions of the microbes?
- It is not clear what the plot sizes for the study of soil invertebrates are, when the company took up the study in the two years [while one was on the campus, the other was during limited field trials, we are made to understand]. The reliability of data from a study like this depends a lot on the plot sizes used because insects and other invertebrates can readily move in and out of small areas. There is little chance of detecting any effects when the plots are small.
- From other studies that looked at soil invertebrates, especially from Bt eggplant crop, it can only be concluded that the impact on non-target invertebrates is not well understood.
- It is also apparent that no comparison has been made with plots which grew non-Bt Brinjal.
- The method of using insect bio-assays for measuring toxin levels in soil samples is unreliable. How do we know that the baseline susceptibility of the larvae chosen is not low? Other methods have to be adopted that would measure the toxin level as well as persistence.
- Finally, what tests have been conducted to assess the impacts of Bt Brinjal cultivation on the next crop – its growth, disease incidence, yields etc., - for medium and long term impacts in a cumulative sense, due to alterations in soil conditions which cannot be captured over just one season?

15. **Toxicity & allergenecity/irritation tests**:

- It is claimed that several toxicity, irritation and feeding tests have been taken up to prove the safety of Bt Brinjal. However, no feedback is possible on the tests since no data has been shared, other than the protocols being shared.

- All that the studies cover are possible acute and sub-chronic [90 days] effects. These tests do not look at long term sub-lethal effects, multi-generational effects, reproductive health effects due to organ damage or effects on growth etc. etc. It is very important that GEAC do not take a decision on a food crop, that too a vegetable crop, without such long term studies.

- The acute oral toxicity test of transgenic brinjal result summary on page 28 of the first document put up on the MoEF website says that the control group was gavaged with non-transgenic cotton seed and not non-Bt brinjal! This is either a typographical error or the fact that the company supplies "set result summary" for various crops, whether Bt Cotton or Bt Brinjal!

- Similarly, feeding tests as in the case of the feeding tests on goats consisted of feeding the animals "with a concentrate of which 12.5% was test seed and the concentrate itself will be 10% of the total feed", whereas Bt brinjal could be fed directly in large quantities [and not just "test seed"] to cattle/livestock especially when there is surplus production and when there is dumping at market yards due to excess production. The cow feeding tests for instance were done with "a total mixed diet where all diets will have the same inclusion level of test/control substance or part of the concentration mixture" with around 2 kgs of fresh transgenic brinjals. When there is dumping in market yards, the consumption could be much higher than this. What tests have been done keeping in mind the worst possible scenario in real life and keeping in mind long term impacts?

- As has been pointed out earlier in the sheep mortality fact finding reports, feeding tests have not been done against sheep [but on goats which are known to be hardier animals] and against real life open grazing conditions. The real feeding conditions also include the fact that they are grazed in open fields, with different parts of the plant consumed, possibly in combination with some pesticide sprays. The other possibility, as in the case of Bt Cotton, there could be misunderstanding amongst farmers that no sprays are required for the transgenic crop and therefore, grazing on the crop is much more safer!

- In the Primary Skin Irritation test done on rabbits, it is not clear what the "test article" was. The animals seem to have been treated with the transgenic vegetable, with two checks of non-transgenic brinjal and untreated check. However, past investigations into the health problems with Bt Cotton have shown that the cotton fibre of the Bt Cotton plant could be inducing the allergic reactions. Similarly, a Filipino study on Bt Maize showed that the pollen could be the allergy-causing agent. How then does a study on the vegetable conclude that workers who work in the Bt Brinjal fields will not be affected [especially given the fact that the reports from various states out that workers are having skin allergy problems while working in bt cotton fields]?

- In the sub-chronic oral toxicity test on rats, it is reported that "There were isolated instances of necropsy findings such as reddening of lungs, dilated kidney pelvis, distended uterus and abscess in salivary gland. The gross pathological changes observed during necropsy were confirmed histologically. The abscess noted grossly in salivary gland was confirmed histologically. Lungs reddening noted at necropsy in four animals, was identified as acute congestion. The incidence of pathological lesions being extremely small, and not dose dependent, was not considered to be of toxicological significance". These findings need more explanation and these could indeed be the 'early warnings' that a precautionary approach requires. The GEAC should ask independent research bodies to conduct the test, with longer periods to find out if this is a finding that requires serious attention.

- Similar are findings related to haematology, clinical chemistry etc., in the case of goat feeding and rabbit feeding studies which can be understood better only if all findings in terms of tabulated numbers are presented.

- There are serious limitations to current allergy testing procedures for GMO proteins. For example, recent results in Australia revealed that a protein previously consumed safely in

beans had become immunogenic (similar to allergic reaction) when engineered into GMO peas. The immunogenicity of the GMO peas would not have been detected by currently used tests. Therefore, new allergy tests, and careful, long-term tests, are needed to assure the safety of Bt brinjal. The pea immunology text is very important because it formally proves that the assumptions underlying the 'event based' approval process are fundamentally wrong. In this test, the Australians also used a latest testing procedure and this paper is annexed to this letter [Annexure 3].

Here, we are also attaching a note on this subject by the world renowned toxicologist, Dr Arpad Puzstai about how the safety testing procedures should ideally be, for various tests [Annexure 4]. Annexure 5 is a note from noted scientists Dr Mae Wan-Ho and Prof Joe Cummins on the serious inadequacies and shortcomings in the biosafety protocols adopted in the case of Bt Brinjal, including the serious implications of not testing beyond *a limiting dose*.

16. **Food Cooking and Protein Estimation studies**: The company claims that studies have been done on protein estimation in cooked transgenic brinjal and reports that the Bt protein was undetectable cooked fruits and that the Cry1Ac protein rapidly degrades upon cooking.

    - ❖ We would like to know if the company or the GEAC have data on how many different ways brinjal is consumed in different parts of the country, by different communities. What does this data say?
    - ❖ The claim that the protein was undetectable and that it degrades rapidly is questionable. While it cannot be detected in its soluble form, what has happened to its breakdown products is important. What are the effects of such products?

17. **On India being the Centre of Origin for Brinjal**: We would like to bring to your notice that ***no GM crop has ever been released in its country of origin so far anywhere in the world***. The overwhelming concerns about a Centre of Origin relate to environmental, agricultural, socio-cultural and IPR issues which have to be given a serious consideration by the regulators. A separate paper is annexed for your ready reference on this subject [Annexure 6] and we reiterate that even the Cartagena Biosafety Protocol to which India is a signatory recommends that a more cautious approach to impact assessment should be made with regard to transgenic crops in their Centres of Origin.

18. **On the socio-economic impact assessments**: There seems to be the presence of a Mahyco member of staff at all times during the interviews which completely invalidates any findings of this survey.

    Where is research on consumer acceptance, consumer willingness to pay for non-GM premiums, on potential effects of markets on farmers etc.? Where is research on socio-economic impacts vis-à-vis successfully established ecological alternatives?

    Where is research on the implications of IPRs on farmers' rights, economics, control over the technology, legal implications and so on, in the socio-economic impact assessment?

19. **Rights of farmers and consumers who wish to be GM-free:** What protection and guarantee is GEAC going to provide for farmers and consumers of this country who have a right to be GM-free and their Right to Safe Food?

Based on this feedback, we demand that the GEAC:

- **respond to our questions and feedback point by point** with all the seriousness that each point deserves

- **show proof** that monitoring and accountability mechanisms are in place and have improved by fixing liability for the violations witnessed so far
- **not** give any permission for large scale trials and seed production or even limited field trials for any GM crop in India until fundamental questions about the decision-making processes related to agricultural technologies are answered and guarantees provided to protect farmers' and consumers' fundamental rights to choices, to safe food etc. and until all the above questions are satisfactorily answered through broad-based public debates that use innovative approaches to include the primary stakeholders in this matter [farmers and consumers of this country and not the company which 'developed' the transgenic brinjal and therefore is staking its ownership claims through patent applications].

Signed & Endorsed by:

	Name	**Designation/Description**	**Organisation**	**State**
1	Dr M S Chari	Entomology expert, Ex-Director, Central Tobacco Research Institute	Managing Trustee, Centre for Sustainable Agriculture	Andhra Pradesh
2	Dr N K Sanghi	Plant Breeder, Ex-Zonal Coordinator, ICAR & Ex-Deputy Director General, MANAGE		Andhra Pradesh
3	Dr J Venkateshwarlu	Soil Science expert, Ex-Director, Central Arid Zone Research Institute		Andhra Pradesh
4	A Ravindra	Agriculture Economist	WASSAN	Andhra Pradesh
5	Dr G V Ramanjaneyulu	Agriculture Scientist	Centre for Sustainable Agriculture	Andhra Pradesh
6	Dr T S V Raghunath	Entomology specialist	Centre for Sustainable Agriculture	Andhra Pradesh
7	Salome Yesudas	Consultant, Gender, Water & Human Nutrition		Andhra Pradesh
8	Malla Reddy	President, AP Rythu Sangam	Vice-President, All India Kisan Sabha	Andhra Pradesh
9	Dr K R Chowdhary	Agronomist	Member, Farmers' Welfare Commission	Andhra Pradesh
10	Kavitha Kuruganti	Sustainable Agriculture Activist	Centre for Sustainable Agriculture	Andhra Pradesh
11	Rajashekar	Agriculture Scientist	South Asian Alliance for Poverty Eradication	Andhra Pradesh
12	Kiran Shakkari	Agriculture Scientist		Andhra Pradesh
13	K Hanumantha Reddy	Journalist & Development activist	Navya Seema Development Society	Andhra Pradesh
14	Dr Uma Shankari	Farmer, Sociology professor & social activist	Rashtriya Raithu Seva Samithi	Andhra Pradesh
15	P Balaram	Development activist	Jana Jaagriti	Andhra Pradesh
16	Dr Rukmini Rao	Women's rights & development activist	Gramya Resource Centre for Women	Andhra Pradesh
17	Kumudini	Coordinator	Stree Shakti Telangana Network	Andhra Pradesh
18	Dr S Jeevananda Reddy	Formerly Chief Technical Advisor - WMO/UN & Expert - FAO/UN		Andhra Pradesh
19	Dr Sagari Ramdas	Director	Anthra	Andhra Pradesh
20	N Madhusudan	Secretary	Yakshi	Andhra Pradesh

21	Prof. Arif A. Waqif		Founder Dean (Retd), School of Management Studies, University of Hyderabad), Director, Research & Chairman, AMDISA Fellowship Committee	Andhra Pradesh
22	Saraswati Kavula	Independent film-maker		Andhra Pradesh
23	S Jeevan Kumar	Human Rights activist	Human Rights Forum	Andhra Pradesh
24	Dr K Balagopal	Human Rights activist	Human Rights Forum	Andhra Pradesh
25	Asha Kachru	Promoting Organic Agriculture as an alternative lifestyle & Gender Advocacy in Agriculture	STRAINATA (women's and farmers' organisation)	Andhra Pradesh
26	Walter Mendoza	Independent Development Worker; Focus on 'strengthening local economies and livelihoods' currently dryland farming		Andhra Pradesh
27	Suryakumar	Banker & concerned citizen	Hyderabad	Andhra Pradesh
28	Dr E Haribabu	Alumnus - IIT-Bombay	Hyderabad	Andhra Pradesh
29	Samyuktha Gorrepati	Textile Designer/cotton handloom enthusiast	Hyderabad	Andhra Pradesh
30	M V Sastri	Development Activist & Economist	Convenor, Centre for World Solidarity	Andhra Pradesh
31	R Murali	Secretary	MARI [Modern Architects for Rural India], Warangal	Andhra Pradesh
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33	Lingaiah	Director	CROPS, Warangal	Andhra Pradesh
34	Vijayalakshmi	Director	Grameena Mahila Mandali, Nalgonda	Andhra Pradesh
35	Venu Madhav	Executive Director	SECURE, Khammam	Andhra Pradesh
36	B Manohar Rao	Secretary	Navajyothi, Medak	Andhra Pradesh
37	Saleem	Executive Director	CONARE, Mahbubnagar	Andhra Pradesh
38	Dr Srinivas	Homoepath	Hyderabad	Andhra Pradesh
39	Vinita Sreepada	Psychologist	Hyderabad	Andhra Pradesh
40	Arun Chandra	Team Leader	Chetna Organic	Andhra Pradesh
41	Bharath Bhushan	Child Rights Activist	Hyderabad	Andhra Pradesh
42	Namrata Vaswani	Asst. professor		Ames IA, USA
43	Varsha Mathrani	Environmental Health Coordinator		Anchorage, AK
44	Jayanthi Reddy	Student		ann arbor, MI
45	Indrani Sistla	Urban Planner		Ashburn, VA
46	Nandu Parimi	Engineer		bedminster, nj
47	Akhila Raman	Engineer		berkeley, ca
48	Nanda Mahashetty	Biochemist	National Institute of Health Sciences	Bethesda, MD
49	S. Anbumani	Postdoctoral Research Associate		Blacksburg, Virginia
50	Michael Muench	Writer		Brooklyn, New York
51	Suryaprakash Kompalli	Student		Buffalo, NY
52	Krishna Adusumilli	Engineer	AID	Carlsbad, CA
53	Priya Ranjan	Research Scientis		College Park
54	Chaitanya Saxena	Graduate Research Fellow	The Ohio State University	Columbus

55	Kalyan	Software Engineer		Dayton, OH
56	PD	Software Engineer		Dayton, Ohio
57	Devinder Sharma	Agriculture & Food Policy Analyst	Forum for Biotechnology & Food Security	Delhi
58	Rakesh Tikait, Yudhvir Singh, Dharmendar Malik		Bhartiya Kissan Union, BKU	Delhi
59	Shalini Bhutani	Concerned Citizen		Delhi
60	Swathi Arjunwadkar		Kalpavriksh	Delhi
61	Malvika Kaul	Senior Editor	Women's Feature Service	Delhi
62	Francis Gonsalves	Lecturer and freelance journalist		Delhi
63	Anuj Grover	Engineer	AID Delhi	Delhi
64	Rajeshwar Ojha	Resource Manager	AID, Asha	Delhi
65	Rupalatha Maddala	Research Associate, Duke Eye Center	AID	Durham, NC
66	Srinivas Rao Chadaram	Research Associate, Duke University Medical Center	Association for India's Development (AID)	Durham, NC
67	Rajitha	Engineer	AID	Fairfax, VA
68	Somu Kumar	Programmer	AID	Fairfax, VA
69	Vandana Mathrani	Student		Flushing, NY
70	Satya N Mishra	Electrical Engineer		Fort Collins, CO
71	Rajasekhar Jammalamadaka	Engineer		Framingham MA
72	Devendra Tolani	Scientist		Gaithersburg MD
73	Arun Gopalan	Software	AID	Gaithersburg,MD
74	Murali Bala	Analyst		Germantown, MD
75	Divya	Student, Baroda		Gujarat
76	Kapil Shah	Organic Farming Activist	Jatan Trust	Gujarat
77	Vanaja	Media	Hyderabad	Andhra Pradesh
78	Pandurang Hegde	Social & Environmental activist	Appiko Andolan	Karnataka
79	Rajni Kumar	Currently, stay-at-home mother	Bangalore	Karnataka
80	Karthik Ramanathan	Researcher	Bangalore	Karnataka
81	Rohan	Social Activist	Bangalore	Karnataka
82	Vishal Sapre	Software Engineer, Bangalore		Karnataka
83	Prasad Naldurg	Researcher, Bangalore		Karnataka
84	Rajagopalan R	Retired IIT Madras Professor, Bangalore		Karnataka
85	Gayathri	Lawyer, Bangalore		Karnataka
86	Somanath Nayak	President	Nagarika Seva Trust, Dakshina Kannada	Karnataka
87	Dr Sharadhini Rath		Centre for Budget and Policy Studies, Bangalore	Karnataka
88	Abhisheka	Artist	Bangalore	Karnataka
89	Prasanna Saligram	Community Worker	Bangalore	Karnataka
90	Shilpa	NGO	Bangalore	Karnataka
91	Ullash Kumar R K	Freelance Journalist	Bangalore	Karnataka
92	Sreelatha Menon			Kerala
93	C Jayakumar	Director	Thanal	Kerala
94	Prof M K Prasad	Retd. PVC of Calicut University	Kerala Sasthra Sahitya Parishad	Kerala
95	Sri John Peruvanthanam	Member	State WildLife Advisory Board	Kerala

96	T P Padhmanabhan,	Environmental Activist	Society for Environment Education in Kerala [SEEK]	Kerala
97	K Ajitha,	Women's rights & development activist	Anweshi Women's Counselling Centre, Kozhikode	Kerala
98	Aleyamma Vijayan,	Director	SAKHI Women's Resource Centre, Trivandrum	Kerala
99	Dr Sreepathy Kajampady		Endosulfan Spray Protest Action Committee, Kasaragod	Kerala
100	Nalini Naik		Protsahan	Kerala
101	S Chandrasekharan Nair	Coordinator	Kerala Farmers Internet group	Kerala
102	Ranjini P R	Sahayathri Trust, Trivandrum	Sahayathri Trust, Trivandrum	Kerala
103	M Gangadharan	President	Paddy Farmers' Forum, Wayanad	Kerala
104	Paul Calvert, Veena M, Anuraja, Mariakutty, Raji, Swapna and Gaspar Melchias	EcoSolutions, Trivandrum	EcoSolutions, Trivandrum	Kerala
105	P R Sreekumar		GREENS, Secretariat, Trivandrum	Kerala
106	Sivaraj		"Uravu Bamboo Resource Centre", Wayanad	Kerala
107	Dr Jaffor Palot,		Malabar Natural History Society	Kerala
108	N Badushah,		Wyanad Environmental Protection Committee, Sulthan Battery, Wyanad	Kerala
109	Sri Pandiyode Prabhakaran,	Secretary	National Farmers' Protection Forum, Palakkad	Kerala
110	Anil		Altermedia	Kerala
111	Robin.C A	Chief Editor	Keraliyam, Thrissur	Kerala
112	C K Sujithkumar	CEDAR, Thrissur	CEDAR, Thrissur	Kerala
113	Dr A Latha	Research Coordinator	River Research Centre, Thrissur	Kerala
114	Purushan Eloor	Convenor	Periyar Malineekarana Virudha Samithi, Kochi	Kerala
115	Ajayan R,	Convenor	Plachimada Solidarity Committee	Kerala
116	Praveenkumar K		"Neythal", Nileshwar, Kasaragod.	Kerala
117	V Resalayyan,		Action Council, Vellarada, Trivandrum	Kerala
118	Geo Jose		National Alliance for People's Movement, Kochi	Kerala
119	V S Nair and all staff		Zero Waste Centre, Kovalam, Trivandrum	Kerala

120	Jacob V Lazer	Civil rights activist	People's Union for Civil Liberties (PUCL), Kochi	Kerala
121	Vinodkumar. P		Maithri, PPO Road, Palakkad	Kerala
122	Sudheerkumar		Kasaragod Environment Protection Committee, Kasaragod	Kerala
123	Jayaprakash		Prakrithi Padana Kendra, Malappuram	Kerala
124	M Mohandas		PROVIDENCE GREEN, Kodakara, Thrissur	Kerala
125	S Raju	Coordinator	Common Birds Monitoring Programme, Trivandrum	Kerala
126	V V Rajasree	Coordinator	CLEAN-Thiruvananthapuram	Kerala
127	Swaroop Roy		Equations, Trivandrum	Kerala
128	Babychen T J		Representing the Organic Farmers and Consumers of the Organic Bazaar, Trivandrum	Kerala
129	Padmasree B Sugathakumari	Poetess, Environmentalist, Trivandrum		Kerala
130	Prof R V G Menon,	Retd. Principal, College of Engineering, Trivandrum	Kerala Sasthra Sahitya Parishad	Kerala
131	Dr Thomas Varghese	Retd Agriculture Scientist, Trivandrum		Kerala
132	Dr C Thankam,	Retd Professor of Botany, College for Women, Trivandrum		Kerala
133	Dr K Saradamoni	Retd Scientist, Indian Statistical Institute, Calcutta		Kerala
134	S Usha	Member	Women and Environment task Force, Asia-Pacific Forum for Women, Law and Development (APWLD), Trivandrum	Kerala
135	Dr S Santhi	Scientist, Trivandrum		Kerala
136	S Anitha	Scientist, Trivandrum		Kerala
137	Dr Manju Nair		Achyuthamenon Centre, SCTIMST, Medical College, Trivandrum	Kerala
138	C R Neelakandan	Environmentalist and Writer, Kochi		Kerala
139	E Kunhikrishnan,	Lecturer	Dept of Zoology, University College, Trivandrum	Kerala
140	Annie Punnose,	Social Activist, Trivandrum		Kerala
141	Seenath Teacher,	Department of Zoology	MES College, Valancherry, Malappuram	Kerala
142	Sri V Harilal,		Kerala Sasthra Sahitya Parishad, Trivandrum	Kerala

143	Deepa P Gopinath	Lecturer	Dept. of Electronics, College of Engineering, Trivandrum	Kerala
144	Kuriachan T D	Senior Technician	Institute for Human Resources Development, Trivandrum	Kerala
145	Prakash P. Gopinath,	J.E.,Track Machines, Southern Railway		Kerala
146	Sridhar R	Campaigner	Save Our Rice Campaign	Kerala
147	Usha Balraman	Professor in Zoology	All Saints College, Trivandrum	Kerala
148	Prof D Thankamoni		College of Engineering, Trivandrum	Kerala
149	Dr P K V Anand	Lecturer	Ayurveda College, Thaikkattusseri, Thrissur	Kerala
150	Adv Daisy Thampi	Advocate	High Court, Kerala	Kerala
151	Monica Cortijo	Housewife		Las Heras, Mendoza, Argentina
152	Dr Sunilam	MLA, MP Assembly	MP Kissan Sangharsh Samithi	Madhya Pradesh
153	Prof Debashis Banerji	Director	Baba Amte Centre for People's Empowerment	Madhya Pradesh
154	Rajeev Baruah	working with organic cotton farmers for more than 14 years	Managing Director, bioRE India Ltd	Madhya Pradesh
155	Aruna Rodrigues	Petitioner, Anti-GMO PIL in Supreme Court of India	Sunray Harvesters	Madhya Pradesh
156	Datta Patil	Executive Director	YUVA-Rural	Maharashtra
157	Dr Nitya Ghotge	Director	Anthra	Maharashtra
158	Kishore Nibrad, Kaikadi Bhojekar		Kissan Majdoor Sanghatana	Maharashtra
159	Vijay Jawandhia		Shetkari Sanghatan	Maharashtra
160	Prajwala Tatte		Shetkari Sanghatan	Maharashtra
161	Damodhar Ughade		Shetkari Sanghatan	Maharashtra
162	Ashatai Tarar		Shetkari Sanghatan	Maharashtra
163	Dr Prerna Barokar		Shetkari Sanghatan	Maharashtra
164	Prof. Pravin Nerkar		Shetkari Sanghatan	Maharashtra
165	Venita Fernandes	Medical & Psychiatric Social Worker	Bombay Catholic Sabha ; Women's Desk	Maharashtra
166	Rajendra Patode	Human Right Activist	National Campaign on Dalit Human Rights, Akola	Maharashtra
167	Manshi	Work with an NGO	National Centre for Advocacy Studies, Pune	Maharashtra
168	Dr Debabrata Roy Laifungbam	Public Health Physician	Centre for Organisation Research & Education (CORE), Manipur	Manipur
169	Lalrindiki Ralte		United Mizoram Grassroot Women, Mizoram	Mizoram
170	John O'Connor	Economist		Montreal

171	Ravi Devarasetty	Software Engineer	Association for India's Development (AID), RTP Chapter	Morrisville, North Carolina, USA
172	Ranitendranath Tagore	graduate student	Asha for Education	New Haven, CT
173	Piyush Mehta	Engineer		Newark, CA
174	Balakrishnan	Engineer		NJ
175	Moon Sen	Post Doctoral Fellow	AID (Association for Indian Development)	Ohio, USA
176	Dr C Shambu Prasad	Innovation Management expert & Associate Professor	Xavier's Institute of Management	Orissa
177	Jagannath Chatterjee	Independent Health Reform Activist		Orissa
178	Mangaraj Panda	Development professional	United Artists' Association, Ganjam	Orissa
179	Debjeet Sarangi		Organic Farming Association of India - (OFAI) Orissa chapter, Bhubaneswar	Orissa
180	Parag Shah	PhD candidate	AID	Philadelphia
181	Vimla Gulabani	Student		Philadelphia, PA, USA
182	Uday Shankar	Biomedical Engineer	Association for India's Development	Pittsburgh, PA
183	Radhika Rammohan	Homemaker	Association for India's Development	Pittsburgh, PA
184	Parul Nisha	P.hD. Student	AID-Pittsburgh	Pittsburgh, PA, USA
185	Pratheesh C Mammen	MSc Student	Salim Ali School of Ecology, Pondicherry	Pondicherry
186	Sathish Sundaram	Engineer	Association for India's Development	Portland , USA
187	Srikanth Srinivasan	Researcher		Portland, Oregon
188	Aparna Chidambaram	Non-Profit Org Volunteer	AID	Portland, Oregon
189	Brunda Kattekola	Engineer	Association for India's Development	Princeton, NJ
190	Gobind Thukral	Journalist		Punjab
191	Shameel	Journalist		Punjab
192	Dr Sucha Singh Gill	Economist		Punjab
193	Dr G S Ghumman	Economist		Punjab
194	Dr Joginder Singh	Economist		Punjab
195	Dr Rajinder Kumar	Human Biologist		Punjab
196	Dr G P I Singh	Community Health Expert		Punjab
197	Dr G S Mouji	Physician & Health activist		Punjab
198	Dr Ashok Goyal	Pharmacologist		Punjab
199	Dr Prem Khosla	Pharmacologist		Punjab
200	Dr Manveer Gupta	Physician, President-IMA, Kotkapura		Punjab
201	Dr Satish Sharma	Physician, State President, National Integrated Medical Association		Punjab
202	Dr Inderjeet Kaur	Physician & Social Activist	Pingalwara Society Chairperson	Punjab
203	Manjeet Singh Kadian	Farmers' leader	BKU (Lakhowal)	Punjab

204	Sukhdev Singh	Farmers' leader	BKU (Ugrahan)	Punjab
205	Harjant Singh	Organic Farmer & social activist	Kheti Virasat Mission	Punjab
206	Umendra Dutt	Social & Environmental activist	Kheti Virasat Mission	Punjab
207	Sukhpreet	Student		Punjab
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210	Kirankumar Vissa	Non-profit Director		Rockville, MD, USA
211	Nisha Kapadia	Student		Sacramento, CA
212	Hozefa Haidery	Architect		San Antonio, Texas
213	Raghavan Jayakumar	Scientist		San Diego, CA, USA
214	Fredrick Cloyd	Student		San Francisco, CA
215	Kiran Vemuri	Hardware Engineer	AID	San Jose, CA, USA
216	Rathnaprabhu Rajendran	Software Engineer	Association for India's Development	SanDiego CA
217	Preeti Kukreja	Student	AID	Silver Spring, MD
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219	G Nammalvar	President	TamilNadu Organic Agriculturist Movement, - Trichy	Tamil Nadu
220	Dr V Jeevanantham	President	TamilNadu Green Movement – Erode	Tamil Nadu
221	K R Jeyaraman	Director	TamilNadu & Pondy Consumers Movement – Thiru Turai Poondi	Tamil Nadu
222	L Anthonysamy	President	TamilNadu Environmental Council – Dindugal	Tamil Nadu
223	Oswald Quintal	State Convener	LEISA Network – Trichy	Tamil Nadu
224	Tamilagan	Advocate	Kaveri Network – Trichy	Tamil Nadu
225	Sheelu	Director	Women Cluster – Chennai	Tamil Nadu
226	Siddamma	Director	Bharathi Trust – Chennai	Tamil Nadu
227	Dhanabalan	Secretary	Kaveri Protection Committee – Keevalur	Tamil Nadu
228	Peer Mohammed	Chairman	FEDCOT – Nager Koil	Tamil Nadu
229	R Selvam	Secretary	Erode District Organic Farmers Network – Erode	Tamil Nadu
230	R Renganathan	Director	TEDE Trust - Chengalpattu	Tamil Nadu
231	V V Giri	Director	Voice Test – Trichy	Tamil Nadu
232	Fr. Arul Selvaraj	Director	SMSSS – Paramakudi	Tamil Nadu
233	Fr. Kennedy	Director	TMSSS – Coimbatore	Tamil Nadu
234	Fr. Starwin	Director	TAOSSS – Trichy	Tamil Nadu

235	Madhu Ramakrishnan	Organic Farmer	Kottur Malaiandi Pattinam – Pollachi	Tamil Nadu
236	A Siva Prakasam	Director	SETHNA Network – Perambalur	Tamil Nadu
237	Thachina Murthy	Working on water-related issues	TVVN, Erode	Tamil Nadu
238	Madhumita Dutta	Working on issues related to corporate crime, pollution and health, occupational health and safety of workers	Corporate Accountability Desk	Tamil Nadu
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240	Shweta Narayan	Community Environmental Monitoring		Tamil Nadu
241	Naveen Kumar	Environmental Engineer, Chennai		Tamil Nadu
242	Lata Ganapathy	Musician/Crafts & Clothing business	International Foundation for Carnatic Music (IFCM), Chennai	Tamil Nadu
243	D.Gurusamy	Secretary	Food First Information & Action Network [FIAN], Madurai	Tamil Nadu
244	Kamala	Banking	Chennai	Tamil Nadu
245	A Gunasekaran	Secretary	Organic Farmers' Association of Arachalur region	Tamil Nadu
246	V Kuppusamy	President	Organic Farmers' Association of Kolathur, Salem district	Tamil Nadu
247	A Narayanan	Managing Trustee	Aranya Trust, Pudukottai	Tamil Nadu
248	Er Duraisamy	President	Jalaspandana, Erode district	Tamil Nadu
249	Dr V Jeevanantham	President	Tamil Nadu Green Movement, Erode	Tamil Nadu
250	M K Bojan	Secretary	Kothagiri Wildlife Society, The Nilgiris	Tamil Nadu
251	S Poongudi	President	Vithu - Women's Seed Savers' Forum, Arachalur	Tamil Nadu
252	Surya Vadivelu	President	Anantham Organisation, Arachalur	Tamil Nadu
253	David Howenstein	Teacher		Tokyo, JAPAN
254	Ranjana Ghosh	An extremely concerned citizen	Research Scientist	USA
255	Kiran Kumar Vissa	Non Profit Director	Association for India's Development	USA
256	Arun Gopalan	Software Engineer		USA
257	Venkata Pingali	Doctoral Student		USA
258	Priya Srikanth	Homemaker	Portland, Oregan	USA
259	Ramkumar Sridharan	Engineer	Santacruz, CA	USA
260	Srinivas K	IT Manager	Reston	USA
261	Akanksha	Software Engineer, NOIDA		Uttar Pradesh

262	Prasoon Agarwal	Software professional, NOIDA		Uttar Pradesh
263	Rev. Qamar Joy Zaidi		SOUP, Allahabad	Uttar Pradesh
264	Ardhendu Chatterjee	Director, Sustainable Agriculture Network spread over 10 dists of WB	DRCSC	West Bengal
265	Chandan Mukherjee	President, Society for Equitable Voluntary Actions (SEVA), Calcutta, West Bengal	Society for Equitable Voluntary Actions [SEVA], Calcutta	West Bengal
266	Anupam Paul	Agriculture Scientist	Kolkatta	West Bengal
266	Sailaja Chadaram	Student at Purdue University		West Lafayette, IN
267	Sharanya Naik		ActionAid India	
268	Dr Walter Fernandes	Director	North Eastern Social Research Centre, Guwahati	Assam
269	Pradeep Esteves		Bangalore	Karnataka
270	Dr Prajit K Basu	Dept. of Philosophy, Univ. of Hyderabad	Hyderabad	Andhra Pradesh

Annexure 1 : Letter submitted by a delegation to the Minister for Environment & Forests, Government of India, with a copy to Chairperson, GEAC on June 15th, 2006

To June 15, 2006

Shri A Raja
Hon'ble Minister for Environment & Forests
Government of India, Paryavaran Bhawan
CGO Complex, Lodhi Road
New Delhi 110003

Respected Sir

Sub: **Bt BRINJAL – BIOSAFETY AND BEYOND**

We are a group of concerned civil society organizations consisting of leading farmers' unions, consumer organizations, organic farming groups, NGOs working on environmental and sustainable agriculture issues, women's groups, members of the medical fraternity etc., representing in turn lakhs of Indians, approaching you to intervene into the matter of Bt Brinjal, which is on the verge of obtaining permission for large scale trials and seed production in this country.

This would be the first time that a GM food crop could be allowed to be released into the open environment for this stage of research. This is also the first time in the world that a GM vegetable crop would be grown with the Bt toxin incorporated into it and consumed with very little or even no processing or cooking. It is not out of place to remind here that it was during large scale trials that Bt Cotton's illegal proliferation began in this country and the regulators only watched with helplessness. Things have not improved an iota since 2001 when such contamination began with Bt Cotton in this country.

There are grave concerns with regard to these various developments and since the Environment Ministy's mandate is to protect the Indian environment and the environmental health of all Indians and since the Ministry constitutes one of the important regulators of GM in agriculture in India [by virtue of the GEAC located in the Ministry and by the presence of the Ministry's representatives in the GEAC] we approach you to seek your urgent positive intervention in the issue.

We would like to begin by stating that while we welcome the fact that GEAC has offered, for the first time more than a decade after GM crop research began in India, to put up data related to findings from biosafety tests on Bt Brinjal, the entire process run was completely unacceptable. The data that was put up, as presentations by M/S Mahyco to the GEAC, is completely inadequate for any intelligent and scientific feedback to be provided. This also showed the world how GEAC takes its decisions. It is clear that a body that should ask basic, scientific questions related to health and environmental implications in addition to socio-economic implications for our farmers, has decided to function as a mere 'bureaucratic approval' body and runs its processes only on such company-produced meaningless presentations. This is a shame to this country, which is a signatory to international conventions like the Cartagena Biosafety Protocol that enshrine the concept of biosafety when it comes to GMOs. To make this farcical process worse, GEAC's own press release put out through the PIB [Press Information Bureau] on May 25th, 2006 says that the company's biosafety findings would be put up under the sub-heading "GEAC Clearance Shortly" on the MoEF's website. Does that mean that the GEAC has already decided on the clearance shortly?

We provide our feedback on Bt Brinjal hereunder. Below, we bring up biosafety issues as well as more fundamental issues beyond biosafety. Much of this feedback should also serve as a feedback on the serious shortcomings of our biosafety regime in general and why there is a need to invoke the precautionary principle on GM crops.

1. **There is no justifiable reason whatsoever for experimenting on and introducing Bt Brinjal [and GM crops in general]:** The GEAC or the DBT [Department of Biotechnology] has no good reason and justification to promote a GM Brinjal in this country. Pest management on Brinjal is being successfully practiced by numerous IPM, NPM and organic farmers with non-chemical, non-GE approaches with very satisfactory results all over the country. Within the ICAR establishment, numerous research projects, including on farmers' fields, show that there are very good, inexpensive and absolutely safe results following non-chemical IPM methods in particular and IPM methods in general. Given such vast experience, why is there no political will to put the control over the technology in farmers' hands? We are attaching to this letter a collection of such experiences [Annexure1] which should provide a way forward for our thinking. We are once again reiterating that for the pest management paradigm to shift in this country, what is needed is political will and not GE-like solutions. We all know that pesticide use in fact has very little to do with pest/disease incidence any more and it has suited the pesticide industry and the regulators/agriculture scientists very well to encourage such a situation so far. To get out of this, we don't need a technology-fix but an alternative paradigm of pest management which empowers the farmers to understand their farm ecology and depend on local resources and sustainable practices for pest management.

   More importantly, there is no crisis with Brinjal production. In fact, due to overproduction, farmers do not get adequate market price.

2. **The science is imprecise and the technology unpredictable – Impact Assessment to be broad in scope:** It is well known that GE is based on imprecise science and is an unpredictable technology as there is little control on where the new genetic construct will lodge within one or more of the target cell chromosomes. It is also well known that tests are not conducted to assess the results from the variety of genes that are inserted along with the desired gene [the markers, promoters, terminators etc. etc.]. Scientists do not understand the mechanisms of GE-induced changes in gene expression in sufficient detail. They do not know what to look for and these things are termed 'unintended effects'. It is for this reason that on a whole range of issues, a great deal of research is required before any outcomes can be predicted in a reasonably assured manner.

   Unlike in other countries, in a country like India where a majority of our livelihoods depend on agriculture, any irrevocable or irreversible change to our agriculture needs to be reasonably sure that the benefits being projected are drawn from sound, long term scientific testing and that risk assessment parameters are broad-based. Elsewhere, risk assessment of GMOs also asks a very pertinent question – "is it [introduction of a GMO] socially and ethically justifiable?". We are annexing a paper on such risk assessment [Annexure 2] so that GEAC might at least now pick up the appropriate framework for risk assessment given that millions of farmers in this country would be affected by your decisions.

3. **India is a Center of Origin and diversity for Brinjal**: Our pool of genetic reserves would inevitably be contaminated and this is extremely dangerous given that we are a Centre of Origin and diversity for Brinjal. We have grown Brinjal for the past 4000 years in this country and it is an extremely popular and widely consumed vegetable. Needless to say, horizontal gene transfer from Bt Brinjal into wild, related species of brinjal has serious implications for the very future of Brinjal research and cultivation in the country. The genetic diversity is important because some of the strains will be naturally resistant to lethal pathogens and

pests that may destroy the crops in the future. Once lost, this lack of diversity can lead to the complete loss of the crop.

If the gene confers an advantage to the wild plants, it will spread in those plants and cause possible harm. This is a very different risk then for the crop itself, since most crops, unlike their wild relatives, cannot survive without cultivation. The U.S. National Academies of Science, in a report in 2004, said that genes that control pests, like Bt genes, have a good chance of giving wild plants an advantage and thereby spreading in the environment. Several published experiments with Bt in rapeseed and sunflower have provided preliminary data that Bt genes can indeed give some wild plants a competitive advantage. If the gene spreads in wild relatives of brinjal, its escape into the environment will likely be permanent. The toxin produced by the gene may then kill insects that feed on the wild plants. These insects, in turn provide food for other organisms such as birds and mammals, which may then suffer harm. For these reasons, it is important to determine the possible harmful effects of the Cry1Ac gene in sexually compatible wild relatives and their ecosystems.

The Cartagena Protocol on Biosafety, the only international law to specifically regulate genetic engineering and GMOs (largely focused on transboundary movement, but whose scope also applies to the use of all GMOs), recognises the importance of centres of origin and diversity, and requires this to be taken into account during the risk assessment. *How has this principle been applied in the case of Bt Brinjal in India?*

4. **Potential environmental hazards with Bt Brinjal:** Existing evidence on environmental hazards with GM crops is enough for a precautionary principle to be invoked regarding their regulation. For instance, it was found in studies that GM crops grown in the UK were not only harmful to beneficial insects like ladybirds but could also indirectly harm other and higher life forms, including mammals, domesticated or wild animals/birds and ultimately man, both in the short- and long-term. The three-year UK farm-scale trials were the largest study ever to evaluate some of the ecological effects of GM crops.

- The assessments about frequency and importance of Horizontal Gene Transfer are premature at present. It is accepted that it [HGT] could have an environmental impact even at a frequency that was approximately trillion times lower than what the current risk assessment literature assumes it to be. The current methods of environmental sampling to capture genes or traits in a recombinant manner are too insensitive for monitoring evolution by HGT. This has serious implications for our bio-diversity, especially given that we are the Centre of Origin for brinjal. With the inevitable contamination of the seed stock, which is certain to take place with GM crops, recovering the original genetic stock will be impossible.

- In the case of pollen flow, it is well known that there is ample opportunity for cross pollination in the case of Brinjal. The rates of natural cross pollination may vary depending on genotype, location, insect activity etc. However, it has been reported that the extent of natural outcrossing is from 2 to 48% in the case of India. Further, it is not clear whether there is enough data on the wild and weedy plants that are either close relatives or have some degree of cross-compatibility with these brinjal varieties. No tests have been done to check for cross-pollination with such relatives. The pollen flow studies on Bt Brinjal in India have been done only in one year [2002, even as the backcrossing programme was on?], in two locations, with reported outcrossing put at 1.46% and 2.7% in these two locations. Such pollen flow studies cannot obviously rely on data from one season and two locations. Studies elsewhere have shown that the likelihood of outcrossing from genetically engineered crops is much higher than in non-engineered crops. For obvious reasons, the same care that is taken for maintaining seed production standards [of 200 metres], has to be applied for the worst case scenario with Bt Brinjal, as a very minimum requirement. In such a case, will Bt Brinjal

farmers, who are mostly small and marginal farmers, be able to conform to such guidelines?

- Further, farmers from various parts of the country are reporting a decline in their soil productivity after growing Bt Cotton. While the regulatory tests related to Bt toxin presence and persistence in the case of Bt Cotton showed that the half-life of Cry1Ac protein in plant tissue was calculated at 41 days [which could then persist in the soil as other studies from elsewhere show], it is not clear how in the case of Bt Brinjal it is non-detectable in soil samples tested. Worldwide, it is generally accepted that Bt toxin does alter the soil micro-biology and that more studies are needed to understand the impact of Bt toxin on soil ecology.

- Have the regulators studied the impact of Bt Brinjal on ecologically sensitive areas like the Eastern and Western Ghats and considered how they would prevent the entry of Bt Brinjal into such ecologically sensitive areas?

- We should also consider a scenario where our predominant pest management strategy relies more and more on one gene – the Bt toxin gene, across crops for a range of pests. Such a monoculture of the gene across crops and varieties is bound to spell doom sooner or later.

- Resistance is already predicted in the target pest and resistance management strategy suggested is a 5% refuge. However, Bt Cotton experience shows that farmers do not follow these resistance management strategies. How will this be done in the case of Bt Brinjal, especially with the farmers being mostly small and marginal? If there are several GM crops grown together, the resistance build up will be faster.

5. **The Bt gene is a known toxin that impacts human health and livestock health adversely**:

   Numerous studies worldwide have raised serious questions about potential health impacts of delta-endotoxins. Key assumptions used as the basis for safety claims have been overturned and several adverse findings suggest that GM foods are unsafe. GM-fed animals had problems with their growth, organ development and immune responsiveness, blood and liver cell formation as well as damaged organs [bleeding stomachs, excessive cell growth, inflammation in lung tissue], sterility problems and increased death rates including among the offspring. Risks are increased by the fact that the genes inserted into GM food not only survive digestion, but transfer into body organs and circulation. Transgenes or their fragments have been found in the blood, liver, spleen and kidneys. Introduction or creation of a new or known allergen or toxin is a potential consequence of genetic manipulation, as experience worldwide shows.

   When Bt Cotton was introduced in India, the same set of tests that are now being applied for Bt Brinjal have apparently been run by the company involved and everything was proclaimed to be safe. However, the human health effects of Bt Cotton in India are being reported from all cotton-growing states. Most farmers and farm workers are experiencing allergies of different kinds. Further, a recent scientific investigation made a clear correlation between the exposure to Bt Cotton and these adverse health effects [copy of the report attached – Annexure 3].

   There were also reports on mortality of sheep after grazing on Bt Cotton recently [copy of the Fact Finding Team's preliminary investigation report attached – Annexure 4]. While there have been no systematic investigations done in other places, there are informal reports however that livestock is being adversely impacted upon grazing on Bt Cotton fields from other places too.

While this is the case with cotton, the consequences with a food crop, that too a vegetable crop which will be consumed quite directly, are unimaginable. Never before in the world has the Bt toxin been introduced into a vegetable crop, where the toxin would be consumed in large quantities and without much processing. We are annexing several scientific papers which point out that Cry1Ac gene [Annexure 5], the Bt gene being used in Bt Brinjal, has many established adverse health impacts. These published, peer reviewed papers by scientists demonstrate that recombinant Cry1Ac protoxin is a powerful immunogen (able to produce an immune response), and when fed to mice, induced antibody responses similar to those obtained with the cholera toxin. Research shows that Cry1Ac actively binds to the inner surface of the mouse small intestine. This contests the often-heard argument that Cry proteins don't affect mammals since they supposedly do not have receptors that bind the truncated toxin in the gut!

The entire infamous episode of Starlink contamination [where Cry9C toxin was used] raises the question of whether other Bt toxins that were supposedly screened might nevertheless be allergens. Scientists accept that without a better understanding of food allergenicity, this question cannot be adequately answered. There are serious limitations to current allergy testing procedures for GMO proteins. For example, recent results in Australia revealed that a protein previously consumed safely in beans had become immunogenic (similar to allergic reaction) when engineered into GMO peas. The immunogenicity of the GMO peas would not have been detected by currently used tests. Therefore, new allergy tests, and careful, long-term tests, are needed to assure the safety of Bt brinjal. Other possible risk issues, such as possible unintended harmful changes in the Bt brinjal plants, can also only be addressed by careful long-term and other testing. We cannot afford to make the mistake committed by Australian regulators who discovered the GM peas case only after almost irreversible field trials.

There are some nutritional and toxicological studies carried out on ingested plant GM DNA which provide information on the potential nature of the hazards of GM foods/feeds. These include: wasteful growth of gut tissues and bacterial proliferation, development of intestinal tumours, depression of the body's immune system, interference with the normal development of vital organs of the body (liver, kidneys, sexual organs, etc.) and reproduction. The seriousness of these effects cannot be overemphasized because the harm will be the most pronounced in the young, the old and in people with intestinal disorders.

A human clinical study carried out and published provides strong evidence of Horizontal Gene Transfer from food to humans. This study showed that fragments of GM DNA were incorporated into the bacteria resident in the gut of human volunteers. Significant amounts of transgenic DNA is found to survive most commercial processing or in the gut of mammals, as per studies in various places.

6. The other genes introduced are toxic too:

Antibiotic resistance: In creating Bt Brinjal, NptII gene has been used as a selectable marker. NptII codes for *kanamycin resistance* and globally, there are serious concerns with antibiotic resistance marker genes for obvious reasons – when there is horizontal gene transfer to gut or soil bacteria, this could spread antibiotic resistance widely. Gene flow, especially to pathogenic organisms, related to antibiotic resistance has been established in past studies. This will imply that disease treatment would be more and more difficult.

The Bt Brinjal also has an aad marker gene. *Streptomycin resistant marker* according to EFSA is a potentially dangerous marker to animals and human beings and should not be used in

the case of GM plants used as food.

Transcriptional activity in human cells with CaMV 35 S: Similarly, use of the CaMV 35 S [cauliflower mosaic virus] promoter, used in creating Bt Brinjal is a matter of concern. Published research shows that the 35S promoter can initiate transcriptional activity in human cells, despite the promoter being a plant-specific one. A scientific paper attached throws further light on this [Annexure 6].

The cauliflower mosaic virus (CaMV), the viral promoter used in Bt Brinjal has similarities with the human hepatitis B virus. As all genomes of living species contain dormant viruses, there is a potential for the CaMV promoter to reactivate them raising concerns related to cancers.

One of the major omissions in present day GM risk analysis is that no attempt has so far been made to investigate the obvious link between GM food and intestinal tumour development. As Dr Arpad Puzstai points out, "full reproductive experiments are required in which the reproductive performance of both male and female rats fed on GM- versus non-GM diets should be monitored for several generations because any problems with reproduction could have disastrous consequences for the environment".

The problems encountered in the study of 'growth factor-like' effects on young rats, was attributed most likely, to the CaMv (cauliflower mosaic virus) viral promoter, a promoter put into Bt Brinjal too. Evidence suggests that the CaMv 35S promoter might be especially unstable and prone to horizontal gene transfer and recombination with all the attendant hazards: gene mutation, cancer, re-activation of dormant viruses and generation of new viruses.

Hazards from GM crops released into the environment may spread more readily through Horizontal Gene Transfer because GM constructs are specifically designed to cross the interspecies barrier.

7. **Past history with corporate research shows suppression of important information:** Monsanto, which is supplying the technology to Mahyco and others in the case of Bt Brinjal, is known from past experience to suppress facts that are unfavourable to the company and its potential markets. A secret study on Bt Maize showed significant harm caused to rats fed on the variety called MON 863. The study shows kidney abnormalities and unusually high levels of white blood cells. What is shocking was that the company then went ahead to conclude that these findings were irrelevant and should not be attributed to Bt Maize even though the rats fed on non-Bt Maize showed no such signs! Given such dubious history, how are the regulators relying on data produced only by the company?

   There is a serious and objectionable conflict of interest in the fact that majority of the tests were undertaken by the company promoting Bt Brinjal [pollen flow studies, Cry1Ac protein expression, baseline susceptibility, protein estimation in cooked fruits, soil analysis, substantial equivalence studies etc. etc.]. Out of the various tests conducted, only four were conducted by public sector institutions. All the tests were funded by the company Where are independent studies to verify the claims of the company?

8. **The tests done here are not adequate – Are we even asking the right questions?** A Public Interest Litigation [PIL] on the lack of rigorous biosafety testing for GMOs in India points out that the current biosafety regime is woefully inadequate in India. A copy of the PIL petition is attached in the form of a booklet [Annexure 7] for ready reference. Often, we do not even have the right questions to ask when testing for safety of GMOs. As pointed out earlier, elsewhere, biosafety regime is inclusive of such pertinent questions as "is this socially

and ethically justifiable?". This requires the testing to be done against other known safer alternatives including ecological/sustainable agriculture practices. However, this was not done in the case of Bt Brinjal. Another paper [Annexure 8] by Dr Pushpa Bhargava way back in 2002 outlines what the biosafety regime should constitute. Going by the set of studies that the company has been asked to do by the regulators, it is obvious that feedback has not been picked up and lessons not learnt.

An annexure provides specific feedback on the biosafety claims on Bt Brinjal [Annexure 9].

9. **The agronomic data unreliable and manipulated:** Going through the Annual Report of the All India Coordinated Research Project – Vegetable Cultivation on ICAR-supervised Bt Brinjal multi-locational trials in 2005-06, it is clear that the data presented is manipulated and unreliable. It is not clear why at least 3 out of the 11 Centres for trials did not report back. The data was not statistically analysed and wrong conclusions were drawn based on skewed averages. It is not clear how some centres could obtain such unbelievably high yields while most of the centres were below average. Is this going to be the situation in real life too for farmers? There is no data at all on pesticide use obtained through the trials though Bt Brinjal is developed ostensibly to reduce the use of pesticides. It is also clear that there were no trials taken up to compare with safer, cheaper, farmer-controlled alternatives like organic brinjal cultivation or NPM or IPM approaches. There was not even a comparison against IPM experience from all over the ICAR establishment from more than 10 years' of work.

10. **Pending liability issues with regard to biosafety violations that need to be addressed first:** Right from the first GM crop trials, there have been many reports from the civil society and the media which pointed out to serious GM regulatory failures in India. This failure was evident both from the company's side [one doing GM research] and from the side of the regulators like the DBT and the GEAC.

    Such failure of enforcement inevitably led to contamination of the supply chain from most field trials even before biosafety [within its limited framework in India] tests were completed. In the case of Bt Brinjal too, investigations of a field trial in Kurnool district of Andhra Pradesh pointed out to the fact that the farmer was allowed to sell the Bt Brinjal in the local market [report attached – Annexure 10]. Even though this was brought to the notice of the regulators, no action towards fixing liability for such serious violations was initiated.

    One of the issues that emerged from investigating the Bt Brinjal trial in Kurnool was the observation by the farmer and his relatives that Bt Brinjal needed to be sold only in the mornings when its color was attractive and that it would change color as the day passed! Farmers' observations about such issues are very sharp and this needs serious and deeper investigation. This was later corroborated by the field assistant employed by Mahyco, in a telephonic conversation.

11. **Economic implications:** There are serious economic implications for farmers if India opts for Bt Brinjal. The assessment of such implications would be clear compared against other choices like staying non-GM as well as promoting organic farming. Even a country like Germany had experienced the enormous employment opportunities provided by the organic approach. In a country which should adopt labour-intensive technologies, organic and other sustainable farming options are a viable proposition. On the other hand, transgenic agriculture would sound a death knell for any attempts related to organic farming in India. Contamination is inevitable and co-existence impossible. This would have serious economic implications for the farmers here. Further, it would clearly negate the efforts being put in by the Government of India to promote organic farming in recent times. This is a clear policy-level contradiction.

12. **Consumer choices and rights:** Transgenic contamination (contamination of the natural environment by GMOs) by more than one method, including wind blown and by cross-pollination is an established fact, beyond dispute and there can be no co-existence between GM and non-GM crops. Segregation even at the physical level is impossible in India. What happens to consumer choices and rights in such a case? Where would be the consumer's right to choose in the case of vegetables, even if we assume that segregation upto an extent is possible and labelling could be made mandatory? Indian vegetable purchases from supermarket shelves are minuscule and obviously, labelling is not going to be an answer here. How do we then provide non-GM brinjal to Indian consumers?

13. **Proprietary Rights of the company**: Even as the ABSPII project was publicising through the media that the technology would be available to the Indian public sector and later to the farmers on a cost-to-cost basis, with Monsanto providing the technology and Cornell University/USAID facilitating the process, Mahyco has informed the GEAC in its presentation on May 22nd that it had applied to the Patent Authority for a patent for "this unique event". This of course will open the floodgates of Indian seed industry to patents, which needless to overemphasise will violate farmers' rights. It will also mean that this technology will not be for everyone, though it is being projected otherwise! The true colors of the corporations behind the development of Bt Brinjal have begun showing.

In conclusion, drawing from the experience with another hazardous technology like pesticides, it is obvious that biosafety and impact assessments are not carried out before irreversible release of the technology into the environment. Very often, experimentation is done at the expense of poor Indians including Indian children as scapegoats. Can India afford to make similar mistakes again?

Given all the above, we demand that:

1 Since the effects of this technology/modified organism are unknown and since these are potentially hazardous, the use of this technology and release of those organisms must wait until the hazards are properly understood and the effects known. **This requires the precautionary approach to be followed.**

2 **Biosafety testing should include testing for medium and long term effects** on the environment and human/animal health, in addition to asking questions on the justification of releasing the GMO into the open environment on social and ethical grounds. For this, the regulators as a beginning, should put together all the available data on safer alternatives, as any environment assessment should, like IPM, NPM, organic etc., and compare Bt Brinjal with such alternatives.

3 Proper biosafety tests should be taken up by **independent and scientifically competent bodies in a transparent manner**. Such tests should be allowed to take appropriate time needed to understand the medium and long term effects instead of being hastened in the pursuit of 'fast-track approvals'.

4 The results of such tests **should be made public** and data published in a manner that it can be closely examined by the scientific community. It shall also be **presented to all primary stakeholders [farmers and consumers]** in a manner that meaningful debates are possible, through for instance, mandatory public notice and public hearings etc.

5 Such reviews and debates should also look at issues beyond biosafety and delve into socio-cultural and political aspects related to GM agriculture, given that millions of our lives and livelihoods depend on agriculture here in India.

6 The GEAC, especially representatives from the Environment Ministry should take on board current scientific data [health and environmental] from elsewhere to understand the potential impact of GMOs and to ask the relevant questions in the Indian context. Based on such

available data, they should lucidly justify why a precautionary principle cannot be invoked straightaway, instead of falling into the trap of the Department of Biotechnology which apparently has only one mandate of promoting GMOs.

In summary, we demand that the Environment Ministry as one of the most important stakeholder-regulators of GMOs in this country play its rightful and expected role in protecting the Indian environment, the health interests of Indians. For this, we demand that the Ministry take a precautionary approach and reject the proposal to permit Bt Brinjal large scale trials in the country.

Sd/- Members of Coalition for GM-Free India

CC: Shri B S Parsheera, Chairperson, GEAC

Annexure 9 [to the June 15th letter to the Minister for Environment & Forests]**:**

Specific feedback to the company's claims on its findings through Bt Brinjal tests and trials:

It is utterly meaningless to comment on the company's claims apparently based on their studies and trials with Bt Brinjal. This is because no protocols are described for the tests nor any numbers or tables presented. However, from whatever's put up on the MoEF's website,:

1 Is there data on how many different ways is brinjal consumed by different communities in India and how it is fed to animals? How has the impact assessment taken this on board? What happens in all those cases where the brinjal is consumed directly, both by human beings and by animals?
2 The tests related to allergenecity and toxicity prescribed as part of biosafety testing are obviously inadequate as the experience with Bt Cotton in India shows. Despite being cleared as safe, Bt Cotton is reported to be causing widespread allergies in cotton growing belts of the country. Therefore, the protocols for such tests need to be re-looked at to capture the real adverse potential and such revised and better protocols applied for Bt Brinjal testing, especially given that it is a food crop with the toxin consumed in large quantities with no or very little processing.
3 Feeding tests done on goats do not capture the potential hazards as goats are known to be hardy animals, compared to sheep for instance. The protocol used in the case of Bt Cotton was to feed goats with cotton seed and the results apparently showed that there is no difference between feeding the goats with Bt Cotton seed and non-Bt Cotton seed. There were no multi-generational feeding tests done. What was not clear however was what the exact research protocol was - how old was the cotton seed, for instance? It is now clear that the tests did not capture the reality of farmers grazing their animals on Bt Cotton plants and not seeds. They also do not in any way predict what could happen with sheep. In the case of Bt Brinjal, there was no change in the testing regime from the Bt Cotton testing regime, despite such valuable lessons emerging from the field and despite this being a vegetable!
4 It is not enough to understand the effect of the Bt gene alone while understanding the impacts on human health and environment. It is important to capture the effects of the other genes transferred too. For this, a set of tests have to be evolved and undertaken.
5 It is surprising that the company says that the Bt toxin rapidly degrades in the soil. Published literature shows that this is not the case. There are many studies that show that Bt toxin can persist in the soil and retain its insecticidal activity. It is in any case known that the half life period of Cry1Ac toxin in plant tissue in the case of Bt Cotton is around 41 days. In such a case, why are the studies done by the company showing that the protein presence was non-detectable? At what stage of the crop was the test done?

6 What is the implication of growing Bt Brinjal in terms of the next crop, given the potential impacts on soil?

7 It is also surprising that pollen flow studies were done for just one year in two locations. Other information from India on pollen flow in Brinjal has results that should make any regulator sit up and take a cautious approach. The protocols used for devising Minimum Standards for Seed Production and Certification should be used here, since they have the worst case scenario built into the framework.

8 Such pollen flow studies should begin by listing out the wild species and related [compatible] species available in India in various regions of brinjal cultivation and check the effect of Bt Brinjal growth on such species, in a controlled environment [and not in farmers' fields]. Where is the data on associated biodiversity [like insects, birds, animals, microbes etc.] which depend on brinjal and its related crops [both wild, related and cultivated] and where are the impact studies on such associated biodiversity?

9 No detailed molecular characterization has been provided by the company. This is important, since we now know that developers cannot control where the transgene insert lands and that DNA rearrangements occur, with the potential to affect the spatial and temporal expression patterns of nearby genes.

10 Bt protoxins differ immunologically from the truncated proteins used for testing purposes. There is evidence that the toxic portion of Cry1A proteins can have a different 3-D conformation depending on whether it is part of the protoxin or in its free state. DNA structurally associated with the protoxin is released during the proteolysis process that generates the toxic fragment from the protoxin. If safety testing was performed on truncated versions of bacterial surrogate proteins rather than the full-length plant-produced Bt proteins that people are actually exposed to, such testing is absolutely inadequte. It has been found often that biosafety testing does not take into account such a difference and it is not clear how the tests were conducted here.

11 It is obvious that investigations have not been carried out to check whether the bacteria in the GM agro-ecosystems have 'picked up' DNA sequence fractions of kanamycin resistance reporter genes or streptomycin-resistance reporter genes.

12 What do the "isolated instances of necropsy" findings in all treatments indicate and what is the company's explanation, in the case of Sub-Chronic Oral Toxicity studies in rats? How many such instances in Bt-treated rats and how many in non-Bt treated?

13 Where is the data on how the Bt Brinjal affects children?

14 Where is the data on the cultural diversity that exists with regard to the cooking of brinjal in this country? Brinjal is also used for medicinal purposes in India. What impact would Bt Brinjal have on such use? Where is data related to socio-cultural importance of Brinjal in different communities in India and the possible impact of Bt Brinjal on the same?

15 Where is data on quantified protein expression related to pest incidence in the complete growing season of the crop? Given that the expression of the toxin is highest in the fruit, the consumed part, what implications does this have for human health for particular hybrids?

16 Deeper investigations into what the farmers have observed during field trials of Bt Brinjal – of color change in the fruits as the day passes – have to be taken up.

17 There is no data that shows that pesticide use does come down with Bt Brinjal – by how much? How does it compare with NPM and organic practices?

FINALLY, WHERE ARE INDEPENDENT RESEARCH PROJECTS BY THE REGULATORS THEMSELVES TO OBJECTIVELY TEST FOR RESULTS ON EACH OF THE ABOVE ISSUES?

Annexure 2:
USE OF BRINJAL IN AYURVEDA AND OTHER TRADITIONAL SYSTEMS OF MEDICINE

Brinjal is one of the important medicinal plant in the traditional ayurveda system of medicine in our country. Different species of brinjal (apart from Solanum melongena which is also used widely as vegetable all over the country) are used in the ayurveda preparations, especially the roots and stem of the plant.

The most required species are given below:

- Solonam xanthocarpum Schrad& wendal. - Dasamoolam (bhaishajyaratnavali) Indukantham ghritham (Sahasrayogam)
- Solanam indicum Linn - Dasamoolam (bhaishajyaratnavali), Dhanwantharam kwadham (ashtangahridayam)
- Solanam nigram Linn - (kantakari) - asthama, rheumatism, heart diseases, hydrophobia, etc It is the major ingredient in Kantakaryavalehyam (Sarngadharasamhitha).
- Withania somnifera - Aswagandha is a major rejuvenative , cardiotonic, effective against neurological complaints, sexual debility, emaciation, etc. It is the major ingredient in Aswagandharishta (ref.Bhishajyaratnavali), Balarishtam (Bhaishajyaratnavali), Aswagandhadi lehyam (Bhaishajyaratnavali)

Other species which comes only in a few formulae and are required in lesser quantity, but a very significant ingredient, are the following:

- Solanam anguivi Lim - (mullan kathiri)- Cardiotonic, skin diseases, diuretic, gynecological disorders
- Solanam melongena Linn - (vazhuthina) - analgesic, aphrodesiac, liver complaints
- Solanam torvum Swartz -
- Solanum incanum Willd -
- Solanam surrattanse Burm.f. (Thudavalam)Rheumatism, Hypertension, rhinopathy, heart diseases
- Solanam trilobatum Linn - cough, bronchitis
- Solanam viarum linn - birth control, hormone supports

Apart from the ayurveda system, in sidha or unani system, brinjal is also widely used in folklore practices and some species are considered as home remedies.

Hence maintaining the purity of the naturally available varieties of Brinjal is important for the Ayurvedic and other Indian systems of medicine.

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Annexure 4: Transgene products and Bt toxins

- a note by Dr Arpad Pusztai, in the context of the Bt Brinjal development in India

The physiological or immunological interactions with vertebrate organisms of *bacterial* versions of cry proteins, or that of the recombinant protein produced in bacteria have been studied. These revealed that the toxin form of Cry1Ac is a potent antigen in mice, following gastric administration. Specific serum IgG and IgM antibodies and locally produced IgA and IgG antibodies to the toxin were detected. The Cry1Ac protein could also be taken up from the intestinal mucosa to be processed in peripheral lymphoid organs. Several human cell cultures, including colonic epithelial and liver cells, demonstrated a number of cytotoxic reactions when exposed to Bt toxins, and immunologic sensitization of farm workers has been well documented. Accordingly, it would be unwise to use Bt toxin-containing foodstuffs in human or animal diets.

Bt toxins remain in existence in ecosystems for long times that helps to build up resistance in target insects, and cause potentially harmful effects on non-target invertebrate species. GM-crops designed for increased pest resistance, such as the Bt toxin crops are not sufficiently selective and specific for their major pests and, by inflicting damage to beneficial insects, they destroy the natural balance between pests and useful organisms. It also must be emphasized that Bt toxins expressed in transgenic plants have never been systematically tested in mammalian or other vertebrate organisms, neither have the effects of the integration of cry genes in vertebrate cells/organisms been studied. Cry proteins bind to the intestinal mucosa, and other cell types and available cell surface receptors in the consumers. Furthermore, the Bt toxins may conceivably enter into complexes with macromolecules in the producer plant or in the gastro-intestinal tract and these may have biological activities and receptor specificities that differ from that of cry toxins or the unattached plant or intestinal macromolecules.

It is also possible that when the transgenic DNA from GM plant food/feed, is taken up, integrated and expressed in the vertebrate organisms, like the alpha-amylase inhibitor gene from beans when expressed in peas may appear in a number of post-translationally modified forms with altered functions and immune/allergenic properties. Intracellular complex formation or other forms of interactions with endogenous gene products may confer unanticipated activities and effects to the cry gene products.

For all the above reasons and because GM crops developed by transgenic splicing techniques present unique and irreversible risks, no new GM crops should be allowed to be cultivated, commercially traded or incorporated into human food or animal feeds unless, as a minimum, it was first shown by the following testing methods that the crop presents no unacceptable harm:

a. the comparison of the GM- and isogenic lines should include investigations with novel and up-to-date analytical techniques, such as proteomic analysis (2D electrophoresis and mass spectrometric analysis of relevant components)
b. a full biochemical, nutritional and toxicological comparison of the *in planta* produced Bt toxin, and not that of a bacterially produced recombinant surrogate, with that of the original whose gene was used for the transformation, must be done
c. microarray analysis of all novel RNA species in the genetically modified plant must be performed
d. full molecular biological examination should be carried out with particular attention to the possiblity of secondary DNA insertions into the plant genome
e. a full metabolomic NMR, etc analysis of the transformed plant is obligatory
f. the stability to degradation by acid or pepsin or other proteases/hydrolases of GM products, foreign DNA, including the gene construct, promoter, antibiotic resistance

marker gene, etc, must be established in the gut of animals *in vivo*, and not *in vitro* as done presently.

g. with GM lectins, including the Bt-toxins the presence/absence of epithelial binding in the gut should also be demonstrated by immunohistology.
h. an investigation of the nutritional, immunological, hormonal properties and allergenicity of GM-crop itself must be carried out on laboratory animals in short- and long-term experiments

Animal testing is but a first step. If the animals did not suffer any health harm, and only then, the results will have to be validated with human volunteers in clinical double-blind, placebo-controlled drug-type tests but keeping in mind that the harm can be most acute in the young, elderly and sick, particularly those suffering from HIV, hepatitis or other viral diseases.

Annexure 5:

GM Brinjal Contains Bt Toxin Linked to Hundreds of Allergy Cases and Thousands of Sheep Deaths

It would be unthinkable and irresponsible to approve the genetically modified brinjal ***Dr. Mae-Wan Ho*** *and* ***Prof. Joe Cummins*** *find no published studies nor experimental details on safety tests in the application for field releases of the Bt brinjal and raise serious questions*

This report has been submitted to Vandana Shiva and others friends in India on behalf of the Independent Science Panel www.indsp.org

Bt brinjal a test case for other GM food crops

The Indian subsidiary of US seeds corporation Monsanto, Maharashtra Hybrid Seed, has developed genetically modified (GM) brinjal resistant to fruit and shoot borer and is applying for large-scale test releases [1]. Brinjal, an eggplant, is widely consumed in India and recognized for its health promoting properties such as reducing serum levels of cholesterol. Field trials of other GM crops, including mustard and potatoes, will follow the brinjal test releases.

The GM brinjal contains the same Cry1Ac toxin from the soil bacterium *Bacillus thuringiensis* as the widely cultivated GM cotton that has been implicated recently in major health controversies in India. Hundreds of farm workers and cotton handlers developed allergic reactions [2] (More illnesses linked to Bt crops, *SiS*30) and thousands of sheep died from toxic reactions after grazing on the post-harvest GM cotton fields [3] (Mass deaths in sheep grazing on Bt cotton, SiS30).

These controversies on the health hazards of Bt crops corroborate findings dating back to the 1980s, which linked Bt bacteria and spores producing a mixture of Cry proteins to allergic reactions [4]. Cry1Ac itself has been identified as a potent systemic and mucosal immunogen [5] and adjuvant comparable to cholera toxin [6]. Thus, not only can the Bt toxin provoke immune reactions to itself, it can also sensitize a person to develop allergies to other components in the diet. At least 12 dairy cows died in Germany after feeding on GM maize containing a gene coding for a protein similar to Cry1Ac [7] (Cows ate GM maize and died *SiS*21).

Cry1Ac is not the only Bt transgenic protein linked to serious health problems. Dozens of villagers fell ill in the south of the Philippines when a Bt maize with Cry1Ab came into flower in 2003, and five have died since [8] (GM ban long overdue, dozens ill & five deaths in the Philippines, *SiS* 29). Illnesses and death associated with numerous other GM crops with different transgenes have been reported in many species. The most dramatic recent example is the severe stunting and premature deaths in the litter of female rats fed GM soya throughout their pregnancy [8], and the debilitating inflammation of the lungs in mice tested with a transgenic pea containing a normally harmless bean protein [9] (Transgenic pea that made mice ill, *SiS* 29).

A comprehensive public enquiry into the health hazards of GM crops is long overdue, as is a global ban while the enquiry is in place. It is unthinkable and irresponsible to release yet another GM crop with a transgenic protein that has already been implicated in so many illnesses and fatalities.

The Report accompanying the application for field release [1] provides such a superficial description of the GM brinjal and unpublished experiments on environmental and health impacts that it would never have passed muster in Europe; which is not to say that Europe's regulatory system is adequate. We concentrate on health impact studies that, according to the company, show Bt brinjal is as safe as non Bt brinjal.

Toxicological studies raise worrying questions

Toxicological studies were all unpublished experiments conducted (except for one) at Intox Pvt Ltd., and amounted to bland assurances that none of the tests caused any toxicity.

However, some statements in the Report should be examined carefully. On p. 7, it states (emphasis added): "Acute oral administration of transgenic Bt brinjal expressing CrylAc protein to Sprague Dawley rats at the *limiting dose* of 5000mg/kg did not cause any toxicity." What exactly is the limiting dose? Does it mean that beyond 5 000 mg/kg the Bt brinjal was in fact acutely toxic? After all, that is equivalent to a person weighing 50 kg eating a medium-size brinjal, which is not unusual.

The next paragraph reports the results of subchronic oral toxicity study, where it states that "the no-observed-adverse-effect (NOAEL) of transgenic Bt brinjal expressing Cry1Ac protein in Sprague Dawley rat, following oral administration for 90 days was found to be more than 1000 mg/kg body weight. This study demonstrates that Bt brinjal expressing Cry1Ac protein is non-toxic to the study animal by oral route."

The designation of "NOAEL" (no-observed-adverse-effect-level) is worrying as it has no scientific precedent. Does that mean doses higher than 1 000mg/kg body weight could be toxic? So, a person weighing 50 kg eating a quarter of a brinjal a day might be putting herself in danger?

The "allergenicity" studies, unpublished and conducted by another company, Rallis India Limited, contained even less details to support the statement of "no differences between the allergenicity or inflammatory characteristics of the 5 brinjal extracts tested including transgenic Bt brinjal and non transgenic brinjal."

The same goes for the "primary skin irritation test", and the "mucous membrane irritation test", both conducted by Intox Pvt. Ltd.

Nutritional studies highly questionable

Another series of "nutritional studies", involved "compositional analysis", which, the company claims, shows that Bt brinjal is "substantially equivalent" to "control brinjal" and thus "the food and feed derived from Bt brinjal will also be substantially equivalent to the food and feed derived from non-Bt counterpart." Again, there are no experimental details given whatsoever.

Compositional studies have long been rejected by the European public as a demonstration of "substantial equivalence", and "substantial equivalence" itself is widely seen as unscientific and unacceptable as a principle of risk assessment [10] (*The Case for a GM-free Sustainable World*).

Another series of unpublished feeding studies with Bt brinjal on fish, chickens cows, goats and rabbits are reportedly, carried out in a variety of companies and institutions, all demonstrating "no significant differences" between Bt and non-Bt brinjal.

In the only case (chickens) where the amount of Bt brinjal eaten is stated, it constituted 5 or 10 percent of the diets. That is equivalent to little more than a mouthful of Bt brinjal at each meal for a human being.

No molecular data

There are no molecular data in the Report to indicate where and in what form the transgenes have inserted into the brinjal genome, and whether the insert has remain stable, which would have been required under the European directive for deliberate release. It is now generally accepted that genetic modification is "event-specific", the transformation causing a lot of collateral mutational damage to the genome including [10-12] as well as the tendency of the integrated insert to be unstable [10-11, 13-15] (*The Case for A GM-Free Sustainable World*; *Living with the Fluid Genome*; Trangenic lines proven unstable; Unstable transgenic lines illegal).

The only molecular information provided is that the Cry1Ac gene is driven by an "enhanced CaMV 35S promoter" (no further details), and two antibiotic resistance marker genes are present: the *nptII* gene coding for neomycin phosphotransferase II (NPTII) (kanamycin resistance) derived from

the prokaryotic transposon Tn5; and the *aad* gene coding for aminoglycoside adenyl transferase (AAD) (spectinomycin and streptomycin resistance) isolated from bacterial transposon Tn7. The *aad* gene is under the control of a bacterial promoter and hence not expressed in Bt brinjal, though it would be fully active in bacteria.

Horizontal gene transfer not considered

There is strong likelihood that the two antibiotic resistance marker genes will spread to pathogenic bacteria in all environments by horizontal gene transfer [16-18] (FAQs on genetic engineering; Recent evidence confirms risks of horizontal gene transfer) and hence exacerbate resistance to antibiotics that are currently used in human and veterinary medicine. Horizontal gene transfer is not considered at all in the Report.

There is evidence that such resistance markers may spread to bacteria in the gut of animals including human beings [19] (DNA in GM food and feed, *SiS* 23), as well as to bacteria in the soil and water [16] simply because DNA does not break down fast enough in all environments.

Conclusion

In conclusion, it would be courting disaster to release yet another GM crop with a transgenic protein that has already been implicated in so many illnesses and fatalities. The company's dossier is highly unsatisfactory and incomplete, and raises some serious safety questions. It can give no comfort to farmers and cotton handlers who have suffered allergic reactions to Bt cotton, nor to farmers who have lost their sheep to Bt cotton.

Instead of approving more GM crops, regulatory authorities in India should start a comprehensive enquiry into the health impacts of Bt cotton and impose a ban on further releases of all GM crops.

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Transgenic Expression of Bean α-Amylase Inhibitor in Peas Results in Altered Structure and Immunogenicity

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The development of modern gene technologies allows for the expression of recombinant proteins in non-native hosts. Diversity in translational and post-translational modification pathways between species could potentially lead to discrete changes in the molecular architecture of the expressed protein and subsequent cellular function and antigenicity. Here, we show that transgenic expression of a plant protein (α-amylase inhibitor-1 from the common bean (*Phaseolus vulgaris* L. cv. Tendergreen)) in a non-native host (transgenic pea (*Pisum sativum* L.)) led to the synthesis of a structurally modified form of this inhibitor. Employing models of inflammation, we demonstrated in mice that consumption of the modified αAI and not the native form predisposed to antigen-specific $CD4^+$ Th_2-type inflammation. Furthermore, consumption of the modified αAI concurrently with other heterogeneous proteins promoted immunological cross priming, which then elicited specific immunoreactivity of these proteins. Thus, transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants possessing altered immunogenicity.

KEYWORDS: α-Amylase inhibitor; transgenic plant; animal model; Th2 inflammation; mass spectrophotometry

INTRODUCTION

Genetically modified (GM) plants are designed to enhance agronomic productivity or product quality and are being increasingly employed in both agricultural and livestock industries (*1*, *2*). Recently, peas (*Pisum sativum* L.) expressing a gene for α-amylase inhibitor-1 (αAI) from the common bean *(Phaseolus* vulgaris L. cv. Tendergreen) were generated to protect the seeds from damage by inhibiting the α-amylase enzyme in old world bruchids (pea, cowpea, and azuki bean weevils) and are currently undergoing risk assessments (*3*−*6*).

The present study was initiated to (1) characterize the proteolytic processing and glycopeptide structures of αAI when transgenically expressed in peas (pea-αAI) and (2) evaluate in an in vivo model system the immunological consequence of oral consumption of pea-αAI. We demonstrate that expression of αAI in pea leads to a structurally modified form of this inhibitor. Employing experimental models, we show that the structural modification can lead to altered antigenicity. These investigations reveal that expression of proteins in non-native hosts can lead to the synthesis of a protein variant with altered immunogenicity.

MATERIALS AND METHODS

Nontransgenic and Transgenic Plants. Seed meal was obtained from nontransgenic peas, genetically modified peas expressing bean α-amylase inhibitor-1 (αAI) (*5*), genetically modified narrow leaf lupin (*Lupinus angustifolius* L.) expressing sunflower seed albumin protein (SSA) in the seeds (SSA-lupin) (*7*), and from nontransgenic Pinto bean. Seeds were ground into fine flour in liquid N_2 using a mortar and pestle. This seed meal was then suspended in PBS (0.166 g meal/mL), homogenized, sieved through a 70 μm mesh, and stored at −70 °C. In some experiments, seed meal homogenates were cooked at 100 °C for 30 min before administration to mice (indicated in text).

Purification of SSA from Transgenic Lupin and αAI from Common Beans and from Transgenic Peas. αAI was purified from the common beans (Pinto and Tendergreen) and transgenic peas and SSA from genetically modified narrow leafed lupin (SSA-lupin) as previously described (*7*, *8*). Purified proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 15−

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25% gradient, 1 mm thick, mini-gel format) and MALDI-TOF mass spectrometry.

Western Immunoblot Analysis. αAI polypeptide composition was determined in protein extracts from common bean and transgenic peas as previously described (*3*). Protein was extracted from seeds with 0.5 M NaCl, 1 mM EDTA, and 0.1 M *N*-tris(hydroxymethyl)methylaminoethanesulfonic acid at pH 7.8. Aliquots of reduced protein (20 μg by Bradford assay) were fractionated by SDS-PAGE and electroblotted onto nitrocellulose membrane. αAI polypeptides were detected with an αAI antiserum from rabbit and goat anti-rabbit IgG conjugated to alkaline phosphatase (*3*). The concentration of αAI in transgenic peas was determined as 4% of total protein as previously described (*3*).

Structural Analysis of Purified αAI from the Pinto and Tendergreen Beans and from Transgenic Peas. Purified αAI from the common beans, Pinto and Tendergreen, and from transgenic peas were analyzed by matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry (MALDI-TOF-MS). The proteins were dissolved in water (approximately 1 μg/μL), and then 1 μL was mixed with 1 μL of matrix solution (saturated sinapinic acid in 50% acetonitrile/0.1% trifluoroacetic acid) on the sample plate of a Voyager Elite MALDI-TOF mass spectrometer (Perseptive Biosystems) and allowed to dry. Spectra were collected in linear mode with myoglobin used for close external calibration (Sigma, Cat. No. M-1882, 16952.6 $[M + H]^+$, 8476.8 $[M + 2H]^{2+}$).

Mice and Intragastric Administration of Seed Meal from Nontransgenic and Transgenic Plants. BALB/c mice were obtained from specific pathogen-free facilities at the Australian National University. Mice were intragastrically administered 250 μL of seed meal suspension (~100 mg/mL) containing either transgenic peas, nontransgenic peas, SSA-lupin, or Pinto bean twice a week for 4 weeks. In some experiments, serum was taken from the mice at the start of the third and fifth weeks during feeding. The serum antibody titers were determined as previously described (*9*).

Mice and Delayed Type Hypersensitivity Responses. BALB/c mice were administered seed meal as described above. Seven days following the final intra-gastric challenge, mice were subcutaneously injected with 25 μL of the appropriate antigen [Tendergreen-αAI, pea α-AI, or lupin SSA (1 mg/mL in PBS)] into the footpad. The positive control [(+) control] is mice immunized by i.p. injection of 200 μL containing 50 μg of Tendergreen-αAI dissolved in PBS with Alum (1 mg/mL) and subsequently receiving 25 μL of purified Tendergreen-αAI (1 mg/mL PBS). The negative control [(−) control] is mice immunized by i.p. injection of 200 μL containing 50 μg of Tendergreen-αAI dissolved in PBS with Alum (1 mg/mL) and subsequently receiving 25 μL of PBS. DTH responses were assessed by measuring the specific increase in footpad thickness using a digmatic calliper (Mitutoyo, Kawasaki, Japan) 24 h following the challenge. Serum was collected on day 14, and antibody titers were determined as previously described (*9*).

Murine Model of $CD4^+$ Th2 Cell-Mediated Inflammation. BALB/c WT mice were administered seed meal as indicated in the text. Seven and nine days following the final intra-gastric challenge, mice were anesthetized with an intravenous injection of 100 μL of Saffan solution (1:4 diluted in PBS). Mice were intubated with a 22 gauge catheter needle, through which purified αAI from Tendergreen bean or transgenic pea (1 mg/mL PBS), or vehicle control (PBS), was instilled. Airway responsiveness (AHR), mucus production, and eosinophilia were measured 24 h following the final intra-tracheal challenge. AHR to methacholine was assessed in conscious, unrestrained mice by barometric plethysmography, using apparatus and software supplied by Buxco (Troy, NY) as previously described (*9*). This system yields a dimensionless parameter known as enhanced pause (Penh), reflecting changes in waveform of the pressure signal from the plethysmography chamber combined with a timing comparison of early and late expiration, which can be used to empirically monitor airway function. Measurements were performed as previously described (*9*). Lung tissue representing the central (bronchi-bronchiole) and peripheral (alveoli) airways was fixed, processed, and stained with Alcian Blue-PAS for enumeration of mucin-secreting cells or Charbol's chromotrope-Haematoxylin for identification of eosinophils as previously described (*9*).

Intragastric Administration of Purified αAI and OVA. Mice were administered 200 μL of affinity purified Tendergreen- or transgenic pea-αAI (5 μg) with ovalbumin (OVA, 1 mg/mL) in a PBS suspension three times a week for 2 weeks. One week following feeding, the mice were intubated with a 22 gauge catheter needle, through which 25 μL of OVA (1 mg/mL PBS), or vehicle control (PBS), was instilled and the $CD4^+$ Th2-inflammation indices determined as described above. Serum was taken from the mice 1 day after the final intra-tracheal challenge, and serum antibody titers were determined as described (*9*).

Antigen Specific $CD4^+$ T-Cell Response. Peribronchial lymph nodes (PBLN) were subjected to pea-αAI or αCD3/αCD28 stimulation as previously described (*9*). In brief, 5×10^5 PBLN cells/mL were cultured with αAI (50 μg/mL) or αCD3 (5 μg/mL)/αCD28 (1 μg/mL) for 96 h. IL-4, IL-5, IFNγ levels were determined in supernatants from stimulated PBLN homogenates by using the OptEIA Mouse IL-4, IL-5, and IFNγ kits (Pharmingen).

Statistical Analysis. The significance of differences between experimental groups was analyzed using Student's unpaired *t*-test. Values are reported as the mean ± SEM. Differences in means were considered significant if $p < 0.05$.

RESULTS

MALDI-TOF-MS Analysis of αAI. To assess the consequences of transgenic expression of the bean αAI in peas, we initially performed a structural analysis of the transgenically expressed protein (pea-αAI). Pea-αAI was compared by Western blot analysis and MALDI-TOF-MS with natively expressed αAI from the common beans, cvs. Pinto (Pinto-αAI) and Tendergreen (Tendergreen-αAI) (collectively termed bean-αAI). Previous studies have shown that bean-αAI is synthesized as a pre-pro-αAI polypeptide that is cleaved following Asn^{77} to form two peptide chains (α and β), both of which are glycosylated and have one or more amino acid residue(s) removed from their C-termini (*8*). This post-translational processing results in major forms of the α and β chains with masses of 11 646 and 17 319, respectively, and minor forms containing alternative glycans (*10*−*12*). Western immunoblot analysis of Tendergreen-αAI and pea-αAI revealed immunoreactive bands in the 11 000−18 000 mass range consistent with the reported structure (*10*−*13*). Detailed comparison of Tendergreen-αAI with pea-αAI revealed differences in the banding profile, suggesting possible differences in the molecular structure of natively and transgenically expressed αaI (**Figure 1A**).

To better resolve the differences between pea-αAI and bean-αAI, affinity purified αAI was analyzed by MALDI-TOF-MS (**Figure 1B**). The mass spectra of Tendergreen-αAI and Pinto-αAI closely matched a previously published spectrum (*10*) of a bean-αAI (*Phaseolus vulgaris* L. cv. Greensleeves) confirming that both Tendergreen- and Pinto-αAI possess similar well-characterized post-translational modifications and very similar relative abundance of minor processing variants (*10*, *11*). Alignment of our spectra with the previously published data (*10*) allowed identification of peaks in the pea-, Tendergreen-, and Pinto-αAI spectra. The major form of the α-chain (11 646 Da) of bean-αAI contains residues 1−76 by cleavage of the pro-protein following Asn^{77}, removal of Asn^{77}, and the addition of sugar residues ($Man_6GlcNAc_2$ at Asn^{12} and $Man_9GlcNAc_2$ at Asn^{65}). Minor forms of the α-chain of bean-αAI differed by having one to three fewer mannose residues resulting in a series of peaks in the MALDI-TOF spectrum that differ by 162 mass units. In contrast, less heavily glycosylated forms dominated for the α-chain of pea-αAI. In particular, an α-chain with two fewer mannose residues (11 322 Da) was the most abundant for pea-αAI but the least abundant for Tendergreen-αAI (**Figure 1C(i)**). A further difference in the pea-αAI spectrum was a series of minor peaks differing from the main α-chain peaks by either

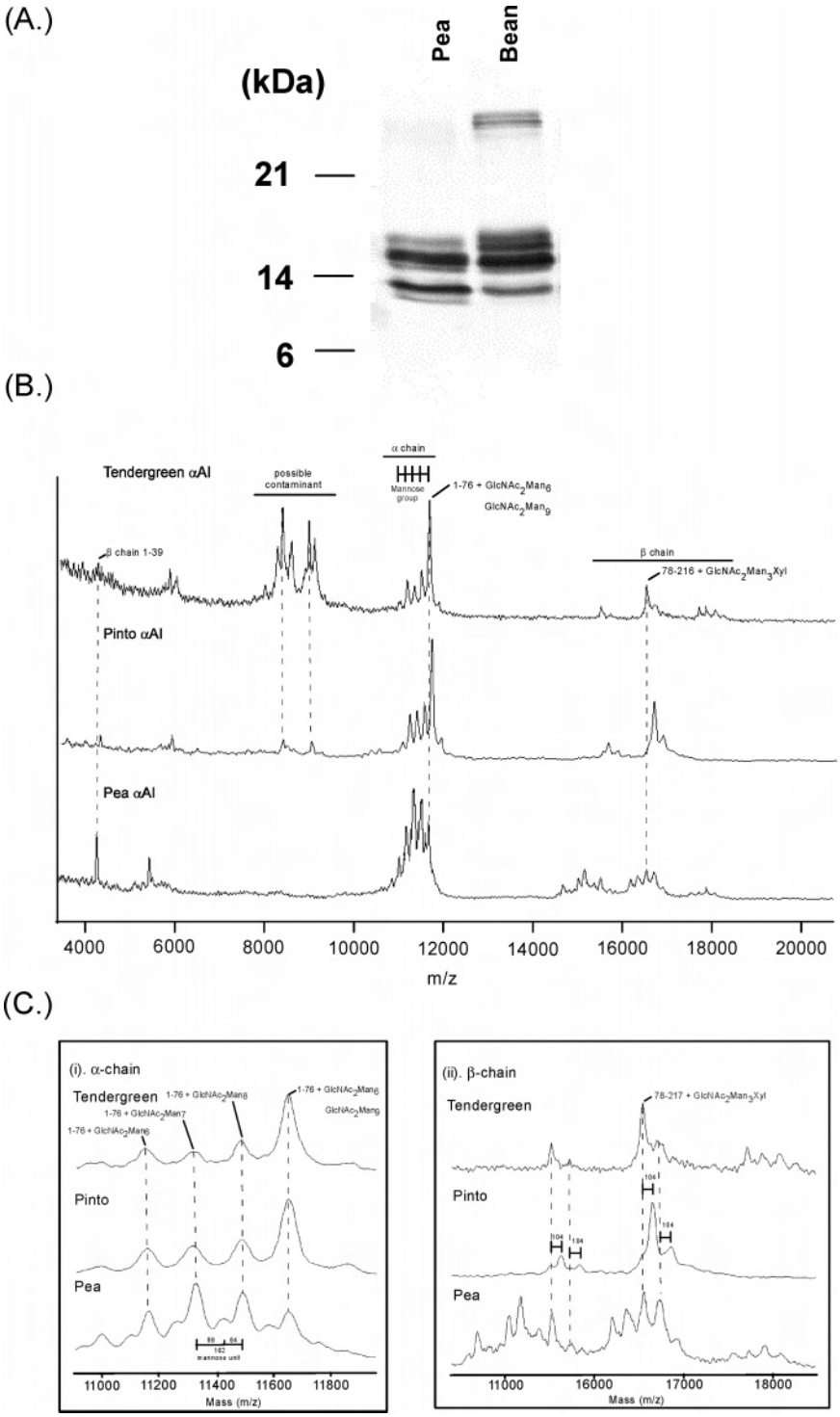

Figure 1. Western immunoblot and MALDI-TOF-MS analysis of common bean-derived-αAIs and αAI from transgenic peas. (**A**) Western blot analysis of αAI protein in extracts of transgenic peas and the Tendergreen variety of common bean. The masses of standard proteins are indicated. (**B**) Aligned MALDI-TOF mass spectra of purified αAI from transgenic pea and the common beans, Tendergreen and Pinto. (**C**) Detail from the spectra in panel B showing the regions of the α-chain (**i**) and the *β*-chain (**ii**).

+98 or −64 mass units, indicating another modification of some of the pea-αAI α-chains (**Figure 1C(i)**).

The major form of the *β*-chain of Greensleeves-αAI (16527 Da) contains residues 78−216 by cleavage of the pro-protein following Asn^{77}, the removal of the seven C-terminal residues following Asn^{216}, and the addition of sugar residues (Man_3-$GlcNAc_2Xyl_1$ at Asn^{140}) (*10*−*13*). The *β*-chain region of the Tendergreen-αAI spectrum closely aligned with that of Greensleeves-αAI (**Figure 1C**). The *β*-chain region of the Pinto-αAI spectrum also closely resembled that of Greensleeves-αAI except that both major and minor peaks of Pinto-αAI were shifted by approximately +104 mass units. This mass discrepancy is consistent with five amino acid residue differences between the *β*-chains of Tendergreen-αAI and Pinto-αAI as predicted by gene sequence comparison (see Supporting Information Figure 1). Further, there are also three predicted residue differences between the Tendergreen-αAI and Pinto-αAI α-chains that result in a difference of +1 mass unit, which would not be

detected by our methods. These sequence differences are consistent with previous reports of αAI polymorphisms among bean cultivars (*12*, *13*). The pea-αAI spectrum showed major peaks corresponding to the two major and minor forms of the *β*-chain found in Tendergreen-αAI; however, the pea-αAI spectrum also showed a number of other peaks (**Figure 1C(ii)**). DNA sequencing of the transgene in pea and comparison with the published sequence (*14*) confirmed that the nucleotide sequences were identical, establishing that the observed further forms of the pea-αAI are related by variations in post-translational modifications including glycosylation (**Figure 1C(ii)**).

Analysis of the spectra of pea- and bean-αAI also revealed several other differences. First, a number of peaks at ~8−9000 and 5824 mass units and below were observed in the bean-αAI spectrum, which are consistent with a previously reported protein that copurifies with bean-αAI (*10*) and doubly charged ($(MH_2)^{2+}$) forms of the α-chain, respectively. Further, a peak at 4223 mass units was detected in the pea-αAI spectrum, which has not been previously reported. While this peak is barely detected in the bean-αAI spectrum presented here, the peak was observed in a number of other bean-αAI preparations (results not shown). The mass of this peak is consistent with the first 39 residues of the *β*-chain, which could be obtained by cleavage following an Asn residue, the same protease specificity that provides the reported processing of αAI at Asn^{77}. Consistent with this hypothesis, a small peak was detected in some preparations at about 12 304 mass units that could correspond to the remainder of the *β*-chain.

While pea-αAI has not yet been characterized as thoroughly as the bean-αAI, it is clear that the transgenic expression of the bean αAI gene in the pea led to differences of glycosylation and possibly other differences in both the α- and the *β*-chains.

Immunological Consequence of Oral Consumption of Beans. Peas are used as a feed component in the livestock industry and also in human diets. Generally, dietary protein antigens undergo gastric digestion leading to the formation of nonimmunogenic peptides and the induction of a state of specific immunological unresponsiveness termed oral tolerance (*15*, *16*). However, the demonstration of structural differences between the transgenic αAI in pea and the natively expressed bean forms raised the concern that the tolerance mechanism may be perturbed, possibly leading to enhanced immunoreactivity.

The induction of oral tolerance results in the failure of the immune system to elicit an active immune response to subsequent exposure to the same antigen in the skin (delayed type hypersensitivity [DTH] response) or lung ($CD4^+$ T-helper [Th_2] cell-mediated inflammation). To examine potential differences in immunological responsiveness following oral consumption, mice were fed Pinto bean, which expresses a native form of αAI and subsequently received purified Tendergreen-αAI in the skin and lung. Most varieties of common beans such as Red Kidney or Tendergreen contain high levels of phytohemagglutinin (PHA), an anti-nutritional factor that induces dietary toxicity in rodents and birds. We therefore used the Pinto variety that contains very low levels of PHA (*17*, *18*) as the appropriate control for oral exposure. Oral consumption of native uncooked Pinto bean seed flour followed by intra-tracheal (i.t.) challenge with Tendergreen-αAI or phosphate buffered saline (PBS) failed to induce an αAI-specific IgG_1 antibody response (**Figure 2A**). Similarly, sub-cutaneous (s.c.) challenge of the footpad or i.t. challenge of Pinto bean-fed mice with Tendergreen-αAI also failed to promote a DTH response (results not shown) or a pulmonary Th_2-inflammatory response [pulmonary eosinophilia, mucus hypersecretion, and enhanced AHR to a bronchoconstrictive agents], respectively (**Figure 2B−D**). While the level of AHR in the Pinto bean-fed αAI-challenged mice was higher than PBS-challenged mice, the level of responsiveness is not significantly different from that of naïve mice i.t. challenged with Tendergreen-αAI (**Figure 2D**). As a positive control, mice were sensitized by intra-peritoneal (i.p.) injection and subsequently challenged via the airways with bean-derived αAI to induce immunological responsiveness (**Figure 2A−D**). Collectively, these data showed that oral consumption of the native bean form of αAI followed by respiratory exposure to bean-αAI did not promote immunological responsiveness or inflammation.

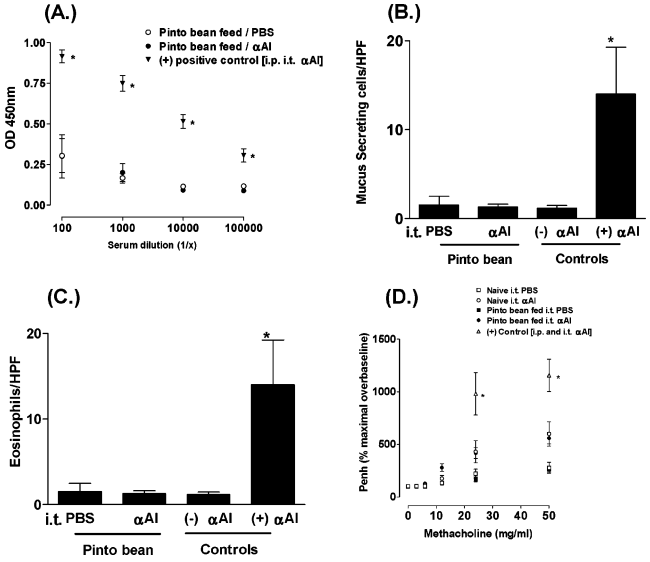

Figure 2. Experimental consumption of bean (cv. Pinto) seed meal does not predispose to inflammation. (**A**) αAI-specific IgG_1 in serum and (**B**) mucus-secreting cell numbers and (**C**) eosinophil levels in lung tissue from Pinto bean-fed mice i.t. challenged with PBS or Tendergreen-αAI. (**D**) AHR in Pinto bean-fed mice i.t. challenged with PBS or Tendergreen-αAI. Data are expressed as the (**A**–**D** and **F**) mean ± SEM and (**E**) mean O.D. of the serum dilution 1/10 ± SEM from 4 to 6 mice per group from duplicate experiments. (**A**–**D**) * $p < 0.05$ as compared to Pinto bean-fed i.t. αAI.

Immunological Consequence of Oral Consumption of Transgenic Peas. To determine whether oral consumption of the transgenic αAI (from pea) elicited an immunological response, mice were orally administered transgenic pea seed meal and αAI; serum antibody titers and DTH responses were examined. Interestingly, in mice that were fed transgenic pea, but not nontransgenic pea, αAI-specific IgG_1 was detected at 2 weeks and at significant levels after 4 weeks of oral exposure (**Figure 3A**). Consistent with the antibody findings, mice fed nontransgenic pea seed meal did not develop DTH responses following footpad challenge with purified pea-αAI (**Figure 3B**). In contrast, mice fed transgenic pea seed meal exhibited a significant DTH response as compared to the nontransgenic pea exposed group when purified pea-αAI was injected into the footpad (**Figure 3B**). As a control for any general effect of genetic modification, we repeated the experiment with material from two other genetically modified plants, lupin (*Lupinus angustifolius* L.) expressing sunflower seed albumin (SSA) [transgenic lupin] (*9*) and chickpeas (*Cicer arietinum* L.) expressing bean derived αAI. Mice were orally administered lupin or transgenic lupin or chickpea or transgenic chickpea seed meal and subsequently footpad challenged with SSA or αAI and DTH responses were examined. In contrast to transgenic pea, mice fed transgenic lupin or chickpea did not develop

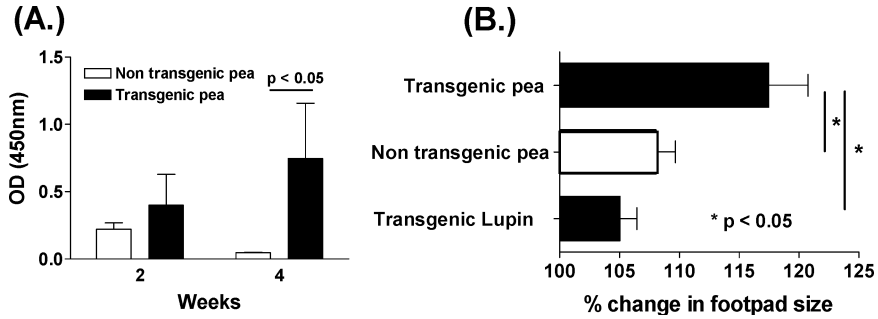

Figure 3. Experimental consumption of transgenic pea seed meal predisposed to antigen-specific IgG_1 and DTH responses. (**A**) Antigen-specific IgG_1 and (**B**) DTH responses in pea nontransgenic and pea transgenic-fed mice. Data are expressed as the (**F**) mean ± SEM and (**E**) mean O.D. of the serum dilution 1/10 ± SEM from 4 to 6 mice per group from duplicate experiments. (**A**–**C**) * $p < 0.05$ as compared to nontransgenic pea or transgenic lupin fed mice i.t. αAI.

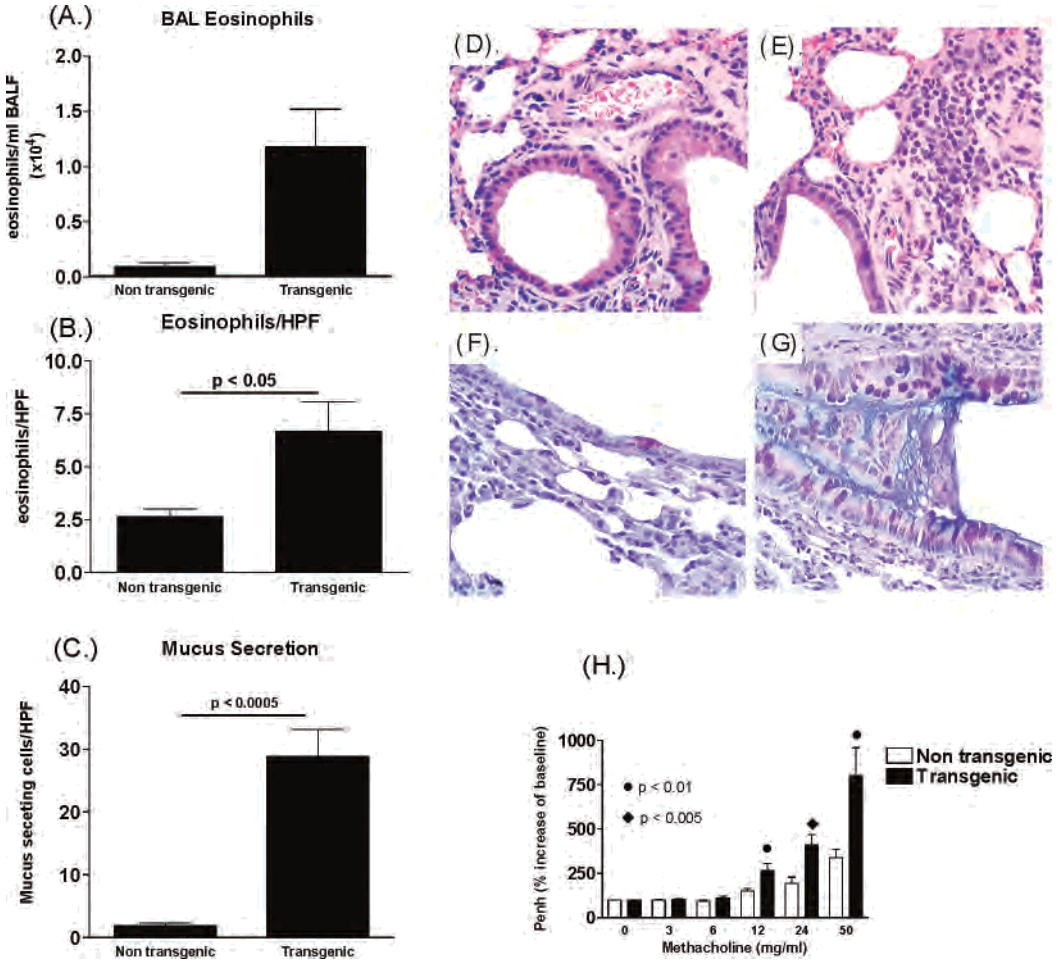

Figure 4. Consumption of transgenic pea seed meal predisposed to $CD4^+$ Th_2-type inflammatory response. Eosinophil accumulation in bronchoaveolar lavage fluid (BAL) (**A**), tissue (**B**), and mucus-secreting cell numbers (**C**) in lung tissue from nontransgenic and transgenic pea-fed mice i.t. challenged with αAI purified from pea. (**D**–**G**) Representative photomicrographs of eosinophil accumulation in lung of (**D**) nontransgenic and (**E**) pea transgenic-fed mice and mucus-secreting cell numbers in lung tissue of (**F**) nontransgenic and (**G**) pea transgenic-fed mice i.t. challenged with αAI from pea. (**H**) Airways hyperresponsiveness (AHR) in nontransgenic and pea transgenic-fed mice i.t. challenged with αAI from pea. Data are expressed as the mean ± SEM from 3 to 6 mice per group from duplicate experiments. Statistical significance of differences ($p < 0.05$) was determined using Student's unpaired *t*-test. (**D**–**G**) ×400 magnification.

DTH responses following footpad challenge with the transgenically expressed and purified SSA or αAI protein (**Figure 3B**; results not shown). Thus, consumption of transgenic pea containing αAI promoted αAI-specific immunological responsiveness.

To characterize the type of immune response elicited against pea-αAI following oral consumption of transgenic pea, we employed a well-characterized murine model of $CD4^+$ Th_2 cell-mediated inflammation (*19*). Mice were orally administered transgenic pea seed meal and subsequently i.t. challenged with purified pea-αAI, and key features of Th_2-inflammation [pulmonary eosinophilia, mucus hypersecretion, and AHR] were examined. I.t. challenge of nontransgenic pea-fed mice with purified pea-αAI failed to induce features of Th_2-inflammation (**Figure 4A**−**G**). Furthermore, airways responsiveness to the cholinergic spasmogen, methacholine, was not induced in these

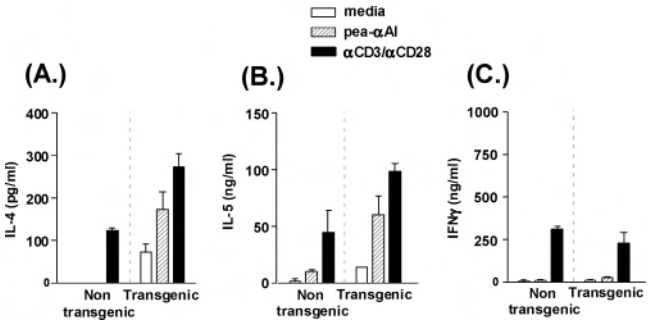

Figure 5. Consumption of transgenic pea seed meal predisposed to $CD4^+$ T-cell derived Th_2-type cytokine production. IL-4 (**A**), IL-5 (**B**), and IFNγ (**C**) levels in supernatants from αCD3/αCD28 or pea-αAI or media alone stimulated PBLN cells from nontransgenic and transgenic pea-fed mice i.t. challenged with αAI from pea. Data are expressed as the mean ± SEM from 3 to 6 mice per group from duplicate experiments. Statistical significance of differences ($p < 0.05$) was determined using Student's unpaired *t*-test.

mice (**Figure 4H**). However, instillation of pea-αAI into the lungs of mice fed transgenic pea induced key features of Th_2-type inflammation including pulmonary eosinophilia, mucus hypersecretion, and AHR (**Figure 4A−H**).

Pulmonary eosinophilia, mucus hypersecretion, and AHR are critically linked to the effector function of the Th_2 cytokines (*20*). To examine whether consumption of transgenic pea promoted a αAI-specific $CD4^+$ Th_2-type T-cell response, $CD4^+$ T-cells in peribronchial lymph node (PBLN) cultures from mice fed nontransgenic pea or transgenic pea seeds challenged with pea-αAI were stimulated with pea-αAI and cytokine profiles determined. Stimulation of $CD4^+$ T-cells in peribronchial lymph node (PBLN) cultures from nontransgenic pea-fed mice challenged with pea-αAI did not elicit Th_2 (interleukin (IL)-4 and IL-5)- or Th_1-type (gamma interferon, IFNγ) cytokine production in response to pea-αAI stimulation (**Figure 5A−C**). By contrast, stimulation of PBLN cultures with pea-αAI from i.t. challenged mice fed transgenic pea resulted in the significant production of Th_2 cytokines (**Figure 5A−C**). Thus, oral exposure of mice to transgenic pea, but not nontransgenic seed meal, predisposed to systemic immunological responsiveness characterized by a Th_2-type immune profile.

Pea-αAI Promotes Immune Responses to Other Oral Antigens. Previous investigations have demonstrated that various plant-derived proteins such as tomatine possess immunomodulatory activity and potentiate and polarize immune responses (*21−23*). We have demonstrated that consumption of transgenic pea in the presence of a large number of potential dietary antigens in the gastrointestinal tract induces an active systemic Th_2-immune response against pea-αAI. In light of these findings, we were next interested in determining whether consumed pea-αAI possessed immunomodulatory activity for Th_2 immune responses and could sensitize mice to heterogeneous nongenetically modified food antigens. Thus, we intragastrically (i.g.) administered purified Tendergreen- or pea-αAI with the well-characterized dietary antigen, chicken egg white protein OVA, or OVA alone and subsequently i.t. challenged mice with OVA. I.g. administration of OVA alone did not systemically sensitize mice to OVA (**Figure 6A**). Further, subsequent OVA challenge in the airways did not promote Th_2-inflammation (mucus hypersecretion, pulmonary eosinophilia, or AHR). Similarly, i.g. administration of bean-αAI and OVA did not systemically sensitize mice or predispose to Th_2-inflammatory processes. However, consumption of pea-αAI and OVA promoted a strong OVA-specific Th_2-type antibody

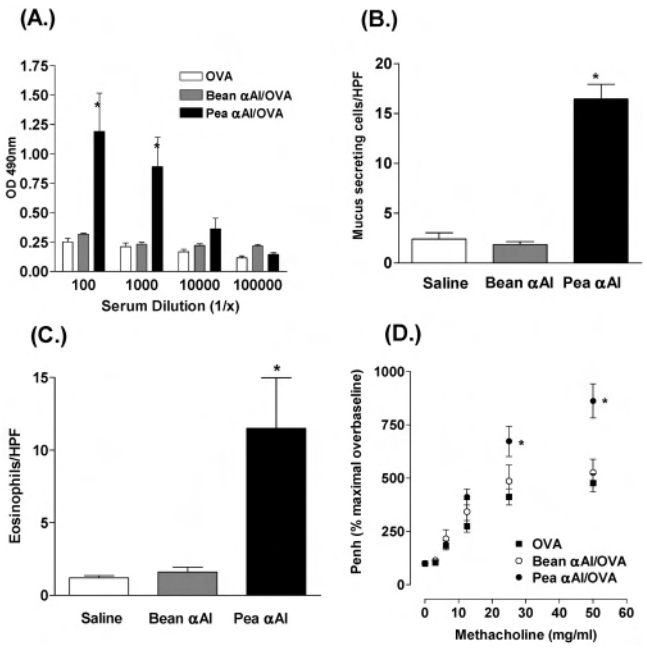

Figure 6. Intra-gastric administration of αAI from pea induces cross-priming of heterogeneous food antigens. OVA-specific IgG_1 levels (**A**) and the Th_2-inflammation phenotype (mucus hypersecretion) (**B**), pulmonary eosinophilia (**C**), and airways hyperreactivity (**D**) in mice that were fed (i.g. challenged) ovalbumin (OVA) alone (the control) or in combination with natively expressed Tendergreen bean-αAI or transgenically expressed (pea) αAI and subsequently intra-tracheal challenged with purified OVA. Data are expressed as the mean ± SEM from 4 to 6 mice per group. * $p < 0.05$ as compared to OVA and bean αAI/OVA.

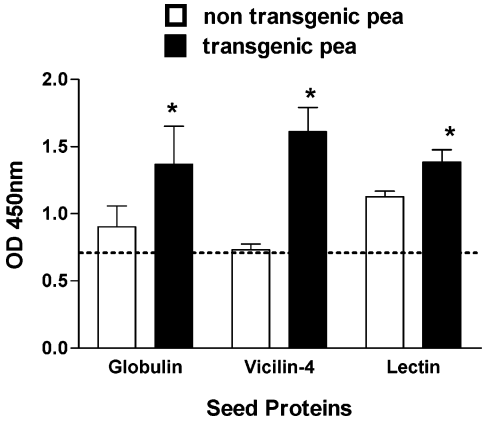

Figure 7. αAI from pea induces cross-priming of pea proteins. Pea globulin-, vicilin-4, and lectin-specific IgG_1 levels in serum from mice that were intragastrically administered 250 μL (~100 mg/mL) of either nontransgenic or transgenic pea seed meal twice a week for 4 weeks. Data are expressed as mean ± SEM from 4 to 5 mice per group. * $p < 0.05$ as compared to nontransgenic pea.

response (**Figure 6A**) and predisposed mice to OVA-induced Th_2-inflammation (**Figure 6B−D**). To support this observation, we examined serum levels of antigen-specific IgG_1 against pea seed proteins (pea globulins, lectin, and vicilin-4) in transgenic pea and nontransgenic pea-fed mice. Interestingly, levels of antigen-specific IgG_1 against pea globulins, lectin, and vicilin-4 in serum of transgenic pea fed mice were significantly higher than those of nontransgenic pea-fed mice, suggesting a heightened immune responsiveness to dietary proteins due to pea-αAI (**Figure 7**). Thus, these studies demonstrate that modified αAI possesses immunodulatory activity and that consumption

of the modified αAI concurrently with heterogeneous proteins can promote immunological cross priming, which predisposes to specific immunoreactivity to these proteins.

DISCUSSION

Recently, peas expressing a gene for αAI from the common bean were generated for protection against field and storage pests (*3*−*6*). Characterization of αAI by structural analysis has demonstrated that transgenic expression of this protein in peas led to the synthesis of a modified form of αAI. Further, we show that the modified form of αAI possessed altered antigenic properties and consumption of this protein by mice predisposed to αAI-specific $CD4^+$ Th_2-type inflammation and elicited immunoreactivity to concurrently consumed heterogeneous food antigens.

Bean-αAI undergoes significant post-translational modification including variable glycosylation and proteolytic processing leading to the synthesis of a mature functional protein (*8*, *11*). We demonstrate that differences in glycosylation and/or other modifications of the pea-αAI lead to altered antigenicity. Consistent with our observations, investigators have previously demonstrated that differential glycosylation of subunits of a cereal α-amylase-inhibitor family (unrelated to legume αAIs) enhances IgE-binding capacity (*24*). Moreover, glycosylated cereal αAI subunits have been shown to possess significantly enhanced IgE-binding affinity when compared to the unglycosylated forms (*24*). These cereal proteins possess identical amino acid sequences and only differ in their carbohydrate moieties, indicating that glycosylation can confer IgE-binding capacity and Th_2-inflammation. In particular, recent investigations have demonstrated that glycan side chains linked to high mannose-type N-glycans on plant-derived glycoproteins can confer immunogenicity and are IgE binding determinants (*25*, *26*). Moreover, α(1,3)-fucose and *β*(1,2)-xylose linkage to high mannose-type N-glycans ($Man_5GlcNAc_2$−$Man_9GlcNAc_2$) promote immunogenicity and IgE binding. The *β*-chain of pea-αAI possesses *β*(1,2)-xylose linked high mannose-type N-glycans, and other complex glycoforms and the α-chain may possess an as yet undefined glycoform variant, and it remains to be determined how these modifications alter pea-αAI immunogenicity.

Functional and structural properties of pea-αAI may contribute to its ability to circumvent immune tolerance and elicit inflammatory responses. Bean-αAI is a potent inhibitor of human α-amylase activity and can induce gastrointestinal dysfunction (*27*). Comparison of bean- and pea-derived αAI activity revealed no difference in enzymatic activity between the two proteins (results not shown). Furthermore, we examined the gastrointestinal tract of pea and transgenic pea-fed mice and observed no histological abnormalities to the gastrointestinal tissue in either group (results not shown). Bean-αAI is also a heat-stable protein and partially resistant to proteolytic degradation (*28*, *29*). Extensive boiling (100 °C for 20 min), while significantly reducing α-amylase inhibitory activity, failed to alter the ability of the transgenic pea to prime for Th_2-inflammation when challenged in the lung [results not shown: see Supporting Information Figure 2]. These findings are consistent with previous demonstrations that cooking of plant material such as lentils and peanuts does not diminish the allergenic potential of certain proteins (*30*, *31*). Furthermore, these studies suggest that the altered immunogenicity of αAI is unrelated to its properties as an amylase inhibitor.

We demonstrate that the immune response elicited against pea-αAI following oral consumption of transgenic pea is characterized by $CD4^+$ Th_2 cell-mediated inflammation, in particular, the presence of IL-4 and IL-5. To examine whether the immune response was dependent on IL-5 and eosinophils, we employed IL-5 and eotaxin-deficient mice. IL-5/eotaxin-deficient mice were i.g. administered nontransgenic and transgenic seed meal and subsequently i.t. challenged with purified αAI. We show that i.t. challenge of transgenic pea fed IL-5/eotaxin-deficient mice induced Th_2-inflammation that was significantly elevated over nontransgenic fed mice (*32*). These investigations suggest that the immune response elicited against pea-αAI following oral consumption of transgenic pea is not dependent on IL-5 and eosinophils.

In this study, we have demonstrated that transgenic expression of αAI in a pea can lead to the synthesis of a modified form of the protein with altered antigenic properties. Furthermore, we show that concomitant exposure of the gastrointestinal tract to modified αAI and heterogeneous food antigens cross primes and elicits immunogenicity. Currently, we do not know the frequency at which alterations in structure and immunogenicity of transgenically expressed proteins occur or whether this is unique to transgenically expressed αAI. These investigations, however, demonstrate that transgenic expression of non-native proteins in plants may lead to the synthesis of structural variants with altered immunogenicity.

ABBREVIATIONS USED

αAI, α-amylase inhibitor-1; pea (*Pisum sativum* L.), transgenic pea; *Phaseolus vulgaris* L. cv. Tendergreen, *Pisum sativum* L. expressing α-amylase inhibitor-1 from the common bean; MALDI-TOF-MS, matrix-assisted laser desorption/ionization-time-of-flight-mass spectrometry.

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Supporting Information Available: Amino acid sequence of αA1 from common bean and consumption of pea seed meal predisposed to Th_2-type inflammation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Controversies in Science and Technology

From Maize to Menopause

Edited by
Daniel Lee Kleinman, Abby J. Kinchy, and Jo Handelsman

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Introduction of Transgenic Crops in Centers of Origin and Domestication

Paul Gepts

In 2003, farmers worldwide planted transgenic crops over about sixty-five million hectares, or 5 percent of total arable land area (James 2003; Food and Agriculture Organization 2004), Most transgenic crops are grown in four countries, the United States, Argentina, Canada, and China. The United States and Argentina together account for nearly 90 percent of transgenic production, with Canada and China accounting for most of the remainder. In the United States, the major transgenic crops are herbicide-tolerant soybean *(Glycine max),* lepidopteran insect-resistant cotton (mainly upland cotton, or *Gossypium hirsutum),* and lepidopteran insect--resistant and herbicide-tolerant maize *(Zea mays);* in Argentina, herbicide-tolerant soybean; in Canada, herbicide-tolerant canola; and in China, lepidopteran-resistant cotton. The shared characteristic of these countries is that none is actually located in the center of genetic diversity and domestication of their respective transgenic crops. Cotton and maize originated in Mesoamerica (Brubaker and Wendel1994; Wilkes 2004), soybean in China (Shimamoto et al. 2000; Li et al. 2001), and oilseed rape presumably in Europe (Sauer 1993). With the exception of oilseed rape, which originated a few centuries ago (Sauer 1993) and has not been fully domesticated yet, the other crops have a history that stretches through several millennia in their respective centers of domestication.

Since Vavilov (1926),[1] it has been known that genetic diversity of crops is unequally distributed across the globe. For many crops, it is possible to identify certain areas with a high level of genetic diversity compared with other areas. Often, these areas correspond also to the center of domestication namely, the process whereby a wild plant is subjected to a selection process conducted under human influence to increase adaptation to cultivated conditions and usefulness to consumers of the harvested products such as grains, fruits, and fibers. Domestication also includes selection for adaptation to new environments, as crops were dispersed from their original centers of domestication to other regions or continents (Gepts 2004a). Many of the domestication centers are actually located in megadiversity centers. Of the seventeen megadiverse countries, at least ten belong to a center of crop domestication (Brazil, China, Colombia, Ecuador, India, Indonesia, Malaysia, Mexico, Peru, and Venezuela).

There is no a priori reason why the introduction in a center of crop domestication of a new cultivar, even a transgenic one, should be cause for alarm. However, several aspects distinguish centers of domestication from other areas where a crop is grown. The different aspects include environmental, agricultural, sociocultural, and intellectual property rights issues. In this chapter, I will discuss each of these aspects and argue that the introduction of transgenic crops into centers of domestication should proceed only with caution, if at all. Many of my examples will address the situation of maize in its homeland (now called Mexico). However, similar arguments can be used for other crops in their respective centers of origin.

Environmental Issues

Gene Flow and Genetic Diversity

The foremost environmental issue is the presence of sexually cross-compatible relatives, whether domesticated or wild. The wild types may he directly related to a crop as progenitors or they may he indirectly related as neighboring taxa. Domesticated relatives are local, farmer selected cultivars, also called landraces. Both wild and domesticated relatives fulfill important roles as reflections of sociocultural identities, production capital of farmers, and repositories of genetic diversity for plant breeders and farmers alike.

An important feature of these domesticated or wild relatives is that they generally cross readily with introduced cultivars. This feature sets the

stage for potentially extensive gene flow in domestication centers between transgenic cultivars and their relatives. On the one hand, crops have evolved to increase self-pollination, which would reduce gene flow among cropvarieties.[2] On the other hand, relatives of transgenic crops may have a more extended flowering time, thus increasing the probability of gene flow. In addition, for animal-pollinated crops, the presence of insect or other animal pollinators that have coevolved with the plant host in centers of domestication may also increase the potential for outcrossing.

Transgenic cultivars present certain issues that arc unique and differ from nontransgenic cultivars in terms of the introduction of transgenes through gene flow. It has often been stated that the transformation process does not carry any inherent risks that do not exist in conventional, sexual transfer. Therefore, the product of the gene transfer, rather than the gene transfer process itself, should be regulated. Carrying this idea to a logical conclusion suggests that certain products of classical plant breeding should also be regulated (Gepts 2002). However, there is a dearth of information about the stability of insertion and expression of transgenes in new genetic backgrounds, especially in centers of domestication where genetic backgrounds may differ considerably from those in which transgenes were originally introduced. It also remains to be seen whether and to what extent this concern extends to nontransgenic cultivars as well.

Gene flow from transgenic cultivars to native materials in centers of domestication has two potential consequences. First is a risk of accumulation of different transgenes in these native materials (called stacking), which may then serve as relays for the unwanted introduction of transgenes to other plant materials, destined fix food or organic production. This could be particularly true for pharmaceutical or industrial compounds, which are highly undesirable in the food chain. However, no foolproof methods yet exist for keeping food and nonfood uses of crops separate. Even in the current seed production systems, transgenes are contaminating nontransgenic seed stocks at a low but measurable level (Friesen, Nelson, and Van Acker 2003; Mellon and Rissler 2004). The problem is even more marked in centers of domestication because the possibilities of physical isolation are more limited, given the presence or sexually compatible relatives. Accumulation of transgenes may also lead to untested combinations of these genes in the same plant.

Second, gene flow may affect the genetic diversity of the landraces and wild relatives in a number of situations. A genetically uniform source

population (such as an improved or hybrid cultivar), high and recurrent levels of migration from the source to the recipient population (i.e., landraces), short distances (depending on the flowering biology of each crop), and/or a combination of these factors can lead to a potentially severe reduction in genetic diversity of the recipient populations and even genetic assimilation (defined as the displacement of the local diversity by the incoming diversity). Transgenic cultivars would not have a monopoly of displacement of genetic diversity. Actually, the development of uniform, elite cultivars by classical breeding has reduced genetic diversity.

The key factor is the degree of uniformity of the improved cultivars. In recent decades the trend has been toward concentration of breeding activities in both the public and private sector. For example, research centers such as the International Maize and Wheat Improvement Center, the International Rice Research Institute, and the International Center for Tropical Agriculture have bred cultivars with wide adaptation that presumably can be grown over broad areas. In the United States, the seed industry has witnessed two rounds of consolidation induced by the availability of molecular biology tools and the application of intellectual property rights to living organisms and basic biological processes. Before this situation existed, breeding programs tended to be smaller and with a more local focus, which maintained a broader range of genetic diversity.

Ecosystem Effects

In addition to concerns about gene flow, it is important to consider that the environments in centers of domestication are quite different from those where transgenic cultivars are grown today, as illustrated by a brief discussion of Bt crops (transformed with the gene for the *Bacillus thuringiensis* [Bt] toxin). Not only are the pests in centers of domestication like Mexico, different, but nontarget organisms (e.g., nonpest lepidopteran and coleopteran species in the case of the Bt toxin) are also quite different. Studies have primarily been conducted in the United States and Europe. For example, following the initial observation by Losey, Rayor, and Carter (1999) of the susceptibility of the monarch butterfly to the Bt toxin, more detailed analyses were conducted (after the regulatory release of transgenic maize), which concluded that the effects of Bt on the monarch butterfly were minimal in the short term in the conditions of the Midwest (Sears et al. 2001 and references therein) but not necessarily in the long term (Scriber 20001). Similarly detailed studies are lacking in

domestication centers, so we do not know the implications for insects in these areas.

Letourneau, Hagen, and Robinson (2002) established a list of about 370 lepidopteran species associated with maize. Of these, only eleven had been examined for their susceptibility to the Bt toxin. Letourneau, Robinson, and Hagen (2003) evaluated what might happen if transgenes escaped into relatives of cotton, rapeseed, and rice by examining lists of sexually compatible relatives, host ranges of lepidopterous insects, their susceptibility to the Bt toxin, and information about the ability of these insects to limit plant growth. They concluded that data are insufficient to establish a risk of ecological release associated with the escape of transgenes among relatives of the three transgenic crops studied. An additional concern may be the effect on certain pollinating, parasitoid, and predator insects (Groot and Dicke 2002). Wolfenbarger and Phifer (2000) present a comprehensive view of the need to measure ecosystem risk and benefits resulting from the introduction of transgenic crops.

Agricultural Issues

Farmers in industrial and in traditional or subsistence agriculture (characteristic of the majority of farmers in centers of domestication) play different roles. In industrial agriculture, farmers have a more specialized role, limited to the production of crops. In contrast, in traditional agriculture, farmers play a role in conservation, development of new cultivars, and processing and consumption of crops in addition to crop production. Specifically, farmers in traditional agriculture play an active role in maintaining crop landraces (in situ conservation; Maxted, Ford-Lloyd, and Hawkes 1997). Landraces are defined as locally distributed and adapted domesticated plants, maintained by fanners. Farmers exert selection to maintain different types according to their use in different cropping systems and for different consumer uses.

Farmers are also willing to experiment by bringing in new materials, including improved bred varieties (e.g., Quiros et al. 1992; Bellon and Berthaud 2004), which then may cross with the local materials and generate new materials (process of *acriollamiento*, or creolization; Bellon and Risopoulos 2001). Farmers exchange seeds with each other, primarily with relatives but also with others in the same or neighboring villages or regions (e.g., Almekinders, Louwaars, and De Bruijn 1994; vom Brocke

et al. 2003; Nkongolo 2003). Through their experimentation and selection, farmers may assure better adaptation of the planting materials to the local agroecological niches. Thus seeds are not merely an agricultural ingredient (like, for example, fertilizer or irrigation water); they are more aptly considered part of the agricultural capital of the farmer, just as land and equipment are.

Genetic diversity is a prerequisite for the development of superior cultivars by farmers and breeders alike. In addition, for farmers genetic diversity is also insurance against the vagaries of production conditions. Typically, a farmer may plant a mixture of cultivars that have different maturities and adaptations to assure some level of production. However, the continued existence of on-farm diversity is threatened by the loss of farmers through migration to cities and other countries, the spread of industrial monoculture cropping systems, and gene flow from or replacement by modern cultivars. Transgenic crops, to the extent that they are an inherent part of industrial agricultural systems, can be a driver in the potential reduction of genetic diversity. Recurrent gene flow from a uniform crop is more likely to displace native genetic diversity, as I mentioned earlier. The combination of intellectual property rights and molecular biology tools has made the development of transgenic cultivars by the private sector possible (Gepts 2004b). Concurrently, the seed industry has been consolidating, so that a few companies now dominate the seed market for several crops, such as maize and cotton. This market concentration raises the possibility that the elite domesticated gene pool will become even more depleted of genetic diversity (Gepts and Papa 2003).

To avoid such a situation in centers of domestication, the transgenic construct could be made available to breeders who could incorporate it into local varieties and thus maintain a more diverse genetic background. Such a situation exists for transgenic herbicide-resistant soybean in the United States. The glyphosate resistance transgene (used in Roundup Ready crop plants) was made available to many companies and public institutions. As a result, a large set of superior cultivars, representing the current diversity in nontransgenic North American soybean, was used to develop current herbicide-resistant cultivars (Sneller 2003). Individual breeding programs, whether public or private, run the risk of having a narrow genetic base. However, public lines originate from many independent programs and as a whole tend to be more representative of the entire range of elite genetic diversity. In addition, current public programs increase diversity through the use of exotic germplasm (Sneller 2003). Exotic germplasm are plant materials that come from different countries or continents. As such, they are generally not adapted to U.S. conditions, but they carry usefu] traits such as disease or pest resistance. The long-term focus of public breeding programs allows them to use exotic germplasm to introduce these useful traits into advanced cultivars. Exchanges among breeding programs are, therefore, essential to maintaining a gene pool of elite cultivars that is as broad-based as possible.

Minimizing reductions in genetic diversity in centers of domestication because of the use of advanced cultivars, whether transgenic or not, would require a diverse group of breeding programs that actively interchange breeding lines. Plant breeding has proved to be a very successful approach that has not lost any of its power. Adoption of modern breeding methods, such as marker-assisted selection, has greatly increased the power of traditional breeding methods. For example, in the common bean, several diseases such as white mold, go]den mosaic virus, and common bacteria, once considered very difficult, if not intractable, are now amenable to genetic improvement through the use of marker-assisted selection, a broader range of germplasm, and improved screening methods (Urrea et al. 1996; Miklas et al. 2001; Kelly et al. 2003). In this respect, transgenic cultivars can make a contribution when screening has shown the native diversity to be insufficient and breeding to improve a critical trait has not worked. An example is the lack of resistance in maize to the European corn borer in the Midwest for which Bt maize provides a solution (Gepts 2002).

However, adoption of improved cultivars may be limited in certain locations. In Mexico, for example, 80 percent of the maize land is still planted with landraces rather than improved cultivars. There are several valid reasons for this limited adoption, including the varied topography (and attendant multitude of microniches), the underfunding of public breeding programs and agricultural research in general, and consumer preference, which is directed to very specific traits, such as colors, textures, cookability, and shelf-life. One solution to this problem might be to decentralize breeding programs to rural areas where farmers themselves would become more involved in the improvement of their local landraces in collaboration with plant breeders (a process also known as participatory plant breeding; Cleveland and Soleri 2002). Such an approach to plant breeding should be part of a broader goal of achieving self-sufficiency in

maize production. In the case of maize in Mexico, A. Turrent (personal communication) has shown that it is possible to raise yield and total production to the point that Mexico becomes self-sufficient for its basic food crop (as well as for its nutritional complement, the common bean).

To make transgenes available-if and when necessary-to a broad section of these programs may require market segmentation for intellectual property rights. This means private companies would have to forgo their royalties for applications in developing countries in order to benefit smallholder farmers, as has been proposed for golden rice (Wai 2003). Another possibility is the public development of transgenic cultivars. The research agenda for transgenic crops should not be determined exclusively by the private sector in industrialized countries. Because the private sector primarily addresses crops with a large market and farmers who can afford to buy seeds, it may not address crops with a smaller market in countries with subsistence farmers. To put transgenic technologies fully to the test, these ought to be designed to fit the agronomic and socioeconomic conditions of smallholder fanners (Chrispeels 2000).

Intellectual Property Rights Issues

One factor driving the development of a transgenic seed industry in the United States and other industrialized countries has been the availability of intellectual property rights over living organisms (Gepts 2004b). The landmark Supreme Court decision in this area was *Diamomd v. Chakrabarty* (447 U.S. 303 [1980]), which set the stage for the award of utility patents for crop cultivars.

The United States is one of only three countries (with Australia and Japan) to award utility patents for crop cultivars. Other countries provide only plant variety protection (PVP) certificates. Utility patents must fit the criteria of novelty, inventiveness, and utility. Unlike the PVP certificates, utility patents do not allow for research exemptions or farmer's exemptions Research exemptions, as allowed by PVP certificates, allow researchers to use patented cultivars as parents in crosses to develop the next generation of improved cultivars. With a farmer's exemption, farmers could harvest patented seeds and replant them on their own land (a practice called seed saving), although they could not sell or give them away to others. Since these exemptions are not allowed by utility patents, developers of genetically modified seeds in the United States have increasingly patented those crop cultivars rather than obtaining the more flexible PVP certificates.

Although utility patents are not available for crop cultivars in most countries, transgenic constructs or methods broadly applicable to plants (i.e., not limited to a specific genotype) are patentable subject matter not only in the United States but in many other countries as well. For example, a transgenic construct carrying the Bt gene or a herbicide resistance gene can be patented. Although a more complete description of intellectual property rights on biodiversity is beyond the scope of this chapter, I do want to note that patent and plant variety protection rights are granted for a limited period (generally twenty years) and a specific place (they are limited to the country that awards them). This being said, patent rights are extremely strong-the courts generally frown on anything that might weaken these rights. For example, patent rights supersede property rights. Ignorance about a patent and lack of intent cannot be used as defense against an infringement accusation. Most surprisingly, gene flow cannot be used as a defense against infringement. Thus, if a company releases a transgenic cultivar, it is not now responsible for the inadvertent escape of transgenes to nontransgenic fields. However, a farmer can be held liable for patent infringement if the patented transgene inadvertently lands on his or her property. This has potential legal implications, especially in centers of domestication where gene flow is particularly widespread.

Although intellectual property rights are limited territorially, their existence nevertheless creates a series of challenges. First, industrialized countries have pushed less-developed countries (where most centers of domestication are located) to adopt intellectual property rights legislation through such mechanisms as the Trade-Related Intellectual Property (TRIPS) agreement, administered by the World Trade Organization (WTO). By joining the WTO, a country commits to the development and enforcement of intellectual property rights legislation. Specifically with regard to crop cultivars, the TRIPS agreement requires countries to provide protection for these cultivars, although not necessarily patenting. The system most often proposed is similar to plant variety protection. Transgenic constructs are still subject to patenting.

The stipulations of intellectual property rights for crop cultivars arc in direct conflict with practices of many farmers in centers or domestication. In traditional agriculture, seed stocks are readily exchanged and arc a public good shared by individuals in communities. This contrasts with individual inventorship and assignment to companies or institutions in industrialized countries. Landraces have been handed down as heirlooms for generations (Zimmerer 1996; Louette and Smale 2000; Perales,

Brush, and Qualset 2003), a practice that also makes identification of individual inventors difficult, if not impossible. Furthermore, many landraces are actually mixtures of genotypes and not pure lines, which would therefore not fit the criteria for plant variety protection. Among the standard practices of farmers are to exchange seed materials and let cross-pollination recombine different genotypes, not only in cross-pollinated species but also in self-pollinated species such as the common bean (Bellon and Risopoulos 2001; Perales, Brush, and Qualset 2003; D. Zizumbo and P. Colunga GarcíaMarín, personal communication). In other words, in traditional agriculture, gene flow is a widely accepted feature or practice, whereas in industrialized agriculture it is to be avoided in order to avoid legal troubles related to intellectual property rights or contamination of the seed stock or grains. Thus introduction of Western intellectual property rights legislation in developing countries creates the possibility that local or indigenous farmers in centers of domestication could be subjected to legal action by the patent holder.

A further consideration is traditional knowledge associated with landraces. Traditional knowledge refers to information held by local or indigenous people, in this case with regard to biodiversity (Brush and Stabinsky 1996). Traditional knowledge is an inherent part of biodiversity and a resource in its own right. For example, Fabricant and Farnsworth (2001) determined that 80 percent of plant-based drugs in Western medicine have had an ethnomedical (i.e., non-western) use identical or related to the current use of the active elements of the plant. With regard to crops, traditional knowledge encompasses information about their agronomic or culinary characteristics. Traditional knowledge is an essential aspect of an indigenous group's cultural survival; it has been developed through generations of intimate contact with the biological materials (Mauro and Hardison 2000). Traditional knowledge is not, however, limited to the knowledge of indigenous people but encompasses knowledge (and associated heirloom varieties) of local, nonindigenous communities in modern societies as well (e.g., Bérard and Marchenay 1996).

Thus indigenous societies or local farmer groups often practice an informal system of innovation and information dissemination, which does not fit well into a Western-style intellectual property rights system, nor does the latter offer rewards for past efforts in innovation and conservation that serve as a foundation for the existence of biodiversity in general and crop biodiversity in centers of diversity in particular. The distinct features of the use and conservation of biodiversity in developing countries have led to a call for a separate legal system that recognizes the contributioms of indigenous or local communities. When dealing with crop landraces, this legal system refers to farmers' rights. However, little progress has been made in developing an enforceable legal framework to support farmers' rights in practice (Gepts 2004b).

Cultural Issues

The long and intimate coexistence of people and crops in centers of domestication is reflected in an extensive cultural presence of the crops among the people, indigenous or not, living in these centers. Maize, for example, has multiple food uses in Mexico, its center of domestication (e.g., Kennedy 2003). Its husks are used as wrapping for dishes, its stalks and leaves as forage, and so on. Mexican Spanish contains an abundance of words derived from pre-Hispanic languages. These words are closely related to the preparation and consumption of maize and attest to the long cultural history of the crop in its center of domestication (Salvador 1997). The importance of basic food crops in their center of domestication is reflected also in their inclusion in creation beliefs. The Popol Vuh (Tedlock 1996), the creation story of the Quiche Maya, relates how, after several failed attempts based on different starting materials, Heart of Sky successfully made humans out of maize. Similar observations can be made for other crops in their respective centers of domestication, such as wheat in southwestern Asia and rice in eastern Asia.

This long-term, close association between people and their respective crops in centers of domestication explains some of their behavior, which at first may seem incomprehensible to outsiders. For example, cultivation of maize in Mexico sometimes takes place despite the lack of economic incentives and returns (Perales, Brush, and Qualset 2003). Rather, non-economic motives such as consumer preferences (color, flavor, cooking quality, shelf life before and after cooking) and cultural identity play an important part as well. Breeding programs, whether they involve transgenic techniques or not, should take these preferences into account. It is not sufficient to consider productivity alone (yield potential, tolerance to abiotic stresses, resistance to biotic stresses). In addition, continued cultivation of maize, a major food crop, can be justified as insurance in the face of uncertain market conditions, which are characterized by uncertain

employment and fluctuating prices, induced in part by international trade agreements.

Emphasis on qualities appreciated by the consumer, in addition to those of importance to the producer, may also be a strategy to assure both the conservation of genetic resources and revenues to the fanner. The European Union has, for example, developed specific designations, such "protected geographic indication" or "protected designation of origin," which could protect local genetic resources and makc their product better known. About five hundred cheese, meat, fruit, and vegetable products are registered as protected geographic indications or protected designations of origin. It remains to be determined whether such attempts at maintaining agricultural and culinary traditions are compatible with the use of transgenic cultivars.

Human and Animal Health Issues

It is beyond the scope of this chapter to address issues related to human and animal health. However, several arguments suggest that these issues need to be addressed in the context of centers of domestication. For example, the genetic composition of human consumers, and therefore the intrinsic reactions to different components included in foodstuffs, may diffcr hom those existing in developed countries such the United States, where transgenic cnltivars have been tested initially. Because some crops are staple crops in centers of domestication, the exposure may vary from that experienced hy human populations in the United States or other countries.

Conclusions

Several issues, including environmental, agronomic, and intellectual property rights, suggest that the introduction of transgenic crops in their respective centers of domestication requires specific attention beyond that devoted to these crops outside the centers of domestication.

A dearth of experimental data often hampers the evaluation of potential risks associated with the introduction of transgenic crops in centers of diversity. Such studies need to he conducted before the introduction of transgenes in domestication centers.

Given several issues that have been raised here, those who want to

introduce transgenic cultivars into a center of genetic diversity and domestication ought to be required to prove that they are safe and can be controlled. There may well be cases in which other approaches, whether genetic or not, will solve the problem while circumventing the issues raised by transgenic cultivars. In turn, these other approaches should also be subjected to comparative risk-benefit analyses.

Delaying or denying the introduction of transgenic crops in centers of origin does not amount to denying the benefits of genetic improvement to the people of these centers. In most cases, classical plant breeding provides a functional alternative that has stood the test of time, although in some limited cases its environmental and human health effects may also need to be monitored.

Transgenic cultivars could playa role if they are specifically designed to address constraints faced by smallholder farmers and fit into the agronomic, environmental, public health, and consumer preferences characteristic of their centers of domestication.

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Notes

1. Nikolai Vavilov (1887-1943), the former director of the All-Union Institute of Plant Industry in St. Petersburg, Russia, was a prominent Russian crop geographer who led countless explorations in Eurasia, Africa, and the Americas. Based on these explorations, he formulated, among others, the theory of the centers of origin of cultivated plants (19926).

2. Plants are characterized by three major reproductive systems. In selfing species (also known as autogamous, or self-pollinating, species), the pollen of a flower is involved in fertilization of the ovules of the same flower. In outcrossing species (also known as allogamous species), pollen is transferred to flowers of other individuals, generally by wind or animals (such as insects or birds). One should keep in mind that the reproductive system of plants may vary to a certain degree. For example, selfing species will generally exhibit some degree of outcrossing, and vice versa for outcrossing species. The transfer of genes, either by pollen or seed, to a new population or location is called gene flow.

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