

Minutes of the 121st meeting of the Genetic Engineering Appraisal Committee (GEAC) held on 18.07.2014

The 121st meeting of the GEAC was held on 18.07.2014 in the Ministry of Environment, Forests and Climate Change (MoEF& CC) under the chairmanship of Shri Hem Pande, Additional Secretary, MoEF and Chairman, GEAC

List of the participants is annexed as Annex 1

Agenda Item No 1 Leave of Absence

The Committee granted leave of absence to Dr S.K. Apte, Dr C. R. Babu, Dr O.P. Govilla and Dr. Vijendra Mishra as requested by him.

Agenda Item No 2: Confirmation of Minutes of the 120th meeting

2.1. Minutes were confirmed with the following editorial changes:

1. Para 4.1.4 line 7 may be re-read as: "TPotpC transit peptide sequences from *Zeamays* and *Helianthus annulus*; others-3' nos and 3' ocs untranslated regions of nopaline and octopine synthase gene of *A. tumifaciens*F".
2. Para 4.2.4 line 1 may be re-read as: "The **Committee** took note of the following documents provided by the applicant."
3. Para 4.2.6, points 1, 3 and 5 may be re-read as follows to:
 1. "Evaluate the efficacy of the BGIII cotton containing Cry1Ac, Cry2Ab2 and Vip3A genes against target lepidopteron insect pests in transgenic cotton corresponding to their conventional counterpart and checks.
 3. Estimate level of expression of Cry1Ac, Cry2Ab2, Vip3A and CP4 EPSPS proteins in various plant parts (terminal leaf, square, boll) at different crop growth stages.
 5. Comparatively assess soil ecosystem, effect on germination, aggressiveness, weediness, morphology and phenotypic characters of transgenic cotton and its conventional counterpart."
4. Para 4.3.2, line 8, the word '**visvidhalaya**' may be re-ad as '**Krish Vishwa Vidyalaya**'.
5. Para 4.8.1 line 2, delete words '**has requested**'
6. The Committee also took note of the following observations made by Dr P.M. Bhargava during the 119th GEAC meeting:

"At this meeting we had an important and long discussion on whether or not we should entertain any applications that relate to the incorporation of herbicide-resistant gene. Many members were strongly of the opinion that such applications should not be

normally entertained as the evidence against the use of herbicide-resistant crops is overwhelming. We, of course, should not stop any research work in the area but we should make it clear that no permission would be granted for field trials of such crops. It was decided that all those who are against the release of herbicide-resistant crops (their names should be mentioned in the minutes) will present evidence in favour of their view at an appropriate meeting where this item would be an agenda item”.

2.2 As regards decision on Agenda item No 4.4 of M/s Pioneer Overseas Corporation to conduct BRL-I trials with 2 transgenic maize P3501YH and 30B07YH (Zea mays L.) hybrids containing *cry 1F and cry 1Ab genes* (stacked events of TC1507 X MON 810), the Committee re-examined the matter on whether it contains only insect resistant trait or both insect and herbicide resistant traits. It was found that it contains both the traits. Accordingly it was agreed that no amendment to the GEAC decision as proposed in the agenda notes would be required.

Agenda item No. 3 Action taken report on the decision taken in the 120thGEAC Meeting held on 12.5.2014.

3.1 The Committee noted that decisions taken in the GEAC meeting held on 12.05.2014 have been communicated to the project proponents, concerned government departments and other agencies.

3.2 As desired by the GEAC, a presentation on the SPT technology was made by M/s E.I. Dupont in the meeting.

Agenda item No 4: Consideration of applications for confined field trials of transgenic crops (Event selection/ BRL-I/ BRL-II) as recommended by the RCGM.

4.1 Permission to conduct event selection trials on five Sugarcane (*Saccharum spp.*) events namely; BtS1, BtS2, BtS3, BtS4 and BtS85 containing *cry1Ac* gene by Sugarcane Research Institute, U.P. Council of Sugarcane Research (UPCSUR), Shahjahanpur.

4.1.1 The Committee considered the request of UPCSUR, Shahjahanpur to conduct event selection trials on five Sugarcane (*Saccharum spp.* CoS 96268) events namely; BtS1, BtS2, BtS3, BtS4 and BtS85 containing *cry1Ac* gene at one location within the The trials will be conducted at one location within the Sugarcane Research Institute Farm at Shahjahanpur in an area of 571 sq m.

4.1.2 The Committee noted that the CoS 96268 (Control/check) is an early maturing high sugar variety of sugarcane having moderate resistance to red rot disease but is susceptible to sugarcane borers.

4.1.3 The Committee also noted that the objectives of the trials are to:

- Assess the expression of *cry1Ac gene* in sugarcane plants.

- Examine further propagation to conform the gene stability in next clonal generation.
- Record the incidence of shoot borer.

4.1.4 The Committee also took note of the field experiment and proposed isolation as given under:

- CoS 96268 is an early maturing sugarcane variety which never flower under subtropical climate. If GM CoS 96268 flowers, then either trial will be terminated or bagging (modified lantern) will be done to check for fertilization/seed formation.
- An isolation distance of 100 m will be maintained as per Minimum seed Certification Standards
- Transgenic sugarcane will be surrounded by lines of the control variety of non-GM sugarcane (CoS 96268) acting as both guard rows of non-GM sugarcane and an isolation zone.
- Post trial land will be used only for genetic transformation experimental purposes. After harvesting, remaining canes will be burnt and disposed properly till the final evaluation and experimentation.
- Two buded setts of GM sugarcane will be planted in the furrow having 2 m plot length with 2 rows each and 90 cm apart between the rows (plot size : 2m x 1.8m), as per recommended practices in sugarcane.
- No herbicide/pesticides will be sprayed/applied throughout the crop season.

4.1.5 The Committee observed the following Information on the gene construct and transformation method:

- (i) Plasmid description: Cry 1Ac gene LBA 4404, EHA 105, carrying the vector PGA492.
- (ii) Transformation: Agrobacterium mediated transformation.

4.1.6 The Committee noted that details of the gene construct as per the format agreed in the GEAC meeting held on 25.04.2014 has not been furnished and opined that the same may be obtained from the applicants in all cases where it has not been furnished.

4.1.7 The Committee also observed that the RCGM had earlier considered the request in its 111th meeting held on 20.03.2012 wherein members observed some discrepancy with respect to the experimental design. RCGM sought clarification/additional information on detailed experimental design for confined field trial on transgenic sugarcane. Revised application containing all the information as mentioned above has been submitted by the applicant.

4.1.8 The Committee observed that the proposal has been recommended by the IBSC and the RCGM in its meeting held on 24.4.2012.

4.1.9 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for conduct of event selection trial on

Sugarcane (*Saccharum spp.*) events namely; BtS1, BtS2, BtS3, and BtS4 containing *cry1Ac* gene by Sugarcane Research Institute, U.P. Council at UPCSR, Shahjahanpur at one location during any appropriate season subject to submission of NOC from the State Government. The Committee also advised the applicant to submit the following information:

- i. Source of Cry 1Ac gene
- ii. Material transfer Agreement
- iii. Target species
- iv. Details of agrobacterium medium
- v. Details of gene construct

4.2 Permission to conduct Biosafety Research Level (BRL-1) trials on insect-tolerant chickpea namely; Bt chickpea 1, Bt chickpea 2, Bt chickpea 3, and Bt chickpea 4 (event SSL-6) containing *Cry2Aa* gene by M/s. Sungro Seeds Limited, New Delhi.

4.2.1 The Committee considered the application of M/s. Sungro Seeds Limited, New Delhi, to conduct BRL-1 trials on insect-tolerant chickpea namely; *Bt chickpea 1, Bt chickpea 2, Bt chickpea 3, and Bt chickpea 4* (event SSL-6 chickpea) containing *Cry2Aa* gene. The trial will be conducted at SAU or ICAR Farm/ Company's own land/ long leased farm in an area of 588.65 sq m. as per details given below:

S.No	Zone	State	Location
1	North West Plain Zone (NWPZ)	Rajasthan	Durgapura
		Haryana	Hisar
2	North East Plain Zone (NEPZ),	Uttar Pradesh	Allahabad
3.	Central Zone	Maharashtra	Rahuri
		Madhya Pradesh	Jabalpur
		Gujarat	Junagadh
4.	South Zone	Andhra Pradesh	Nandyal
		Karnataka	Dharwad

4.2.2 The Committee also noted that the transgenic chickpea expressing *Cry2Aa* gene is similar in phenotype to its non-transgenic counterpart except for resistance to pod borer insect (*Helicoverpa armigera*) of class Lepidoptera.

4.2.3 The Committee noted that the objectives of the trials are to:

- Evaluate the field infestation levels of target insect pest, pod borer (*Helicoverpa armigera*), on transgenic Bt chickpea entries, non-Bt counterparts and checks.
- Estimate the level of expression of Cry2Aa protein in various plant parts at various crop growth stages of the Bt chickpea entries. The protein expression data of Cry2Aa in various plant parts will be recorded at an interval of 30 days, starting from 30 days after sowing (DAS) at all trial locations.
- Observe with respect to growth habit, life cycle, plant height, impact on pollinator species of Bt chickpea entries, their non-transgenic counterparts and checks and any indicators of changes in weediness potential in the Bt chickpea entries.
- Monitor the occurrence of beneficial arthropods and insect pests on Bt chickpea entries, their non-transgenic counterparts and checks.
- Observe the effect of Cry2Aa protein on soil microflora, earthworms and soil insects (*Collembola* spp.) present in the soil rhizosphere collected from Bt chickpea and other non-transgenic chickpea plots.
- Estimate the grain yield of Bt chickpea entries, their non-transgenic counterparts and checks.
- Generate the plant material of Bt chickpea entries, their non-transgenic counterparts and checks for biosafety studies.

4.2.4 The Committee also took note of the field experiment and proposed isolation as given under:

- Randomized Complete Block Design with 3 replications
- Five meters of non-transgenic chickpea will be planted around the trial plot. Apart from these trap rows, isolation distance of 5 m as prescribed for chickpea crop in the Minimum seed Certification Standards will be maintained.
- The trial location will be fenced and demarcation flags will be placed at the boundaries.
- Management of insect pests in the trial will be done by spraying insecticides, based on economic threshold levels of the pest. ETLs of two major pests viz., pod borer and aphids are 1-2 larva/m row length and 1-2 aphids/leaf, respectively.

4.2.5 The Committee also noted that approximately 1 kg/entry/location from three entries (transgenic; non-transgenic counterpart; and non-transgenic reference) from three locations of Chickpea grains will be harvested for compositional analysis.

4.2.6 The Committee observed the following information on the gene construct and transformation method:

Transformation method: Agrobacterium mediated transformation

Detail of Plasmid	Events
The name of the plasmid vector used for transformation	pBK201 vector

What are the promoter and terminator used for expressing the gene of interest/s?	The promoter used for expressing <i>Cry2Aa</i> gene is <i>Arabidopsis</i> SSU (Rubisco small sub unit) promoter and terminator is tobacco SSU terminator.
The antibiotic resistance marker used in transformation (inclusive of promoter and terminator).	The antibiotic marker used during the transformation process was neomycin phosphotransferase II (<i>npt II</i>) conferring resistance to kanamycin. The promoter used in the cassette is a SCSV 1 (Subterranean clover stunt virus) promoter and the terminator is a SCSV 3 terminator.
Are additional antibiotic resistance markers present in the transgenic plant?	No additional antibiotic resistance markers are present in the transgenic plants
Is any reporter gene such as GUS or GFP present in the transgenic plant	No
Is the plant a marker free transgenic?	No, the plants are with <i>nptII</i> marker gene (Kanamycin).

4.2.7 The Committee also observed that the proposal was recommended by IBSC and RCGM in its meetings held on 9.9.2012 and 28.8.2012 respectively.

4.2.8 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for conduct of BRL-1 trials on insect-tolerant chickpea namely; Bt chickpea 1, Bt chickpea 2, Bt chickpea 3, and Bt chickpea 4 (event SSL-6) containing *Cry2Aa* gene at SAU or ICAR Farm/ Company's own land/ long leased farm during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted. The Committee also advised the applicant to submit details of the source of gene.

4.3 Permission to conduct Biosafety Research trials (BRL-II) with two transgenic Bt Brinjal hybrids namely Janak and BSS-793 Bt, containing *Cry1Fa1* (Event 142) gene M/s. Bejo Sheetal Seeds Pvt. Ltd., Jalna.

4.3.1 The Committee considered the request of M/s. Bejo Sheetal Seeds Pvt. Ltd., Jalna, to conduct BRL-II trial with two transgenic Bt Brinjal hybrids namely; Janak and BSS-793Bt, containing *Cry1Fa1* gene (Event 142) at seven locations namely Maharashtra, Gujarat, Punjab, Haryana, Uttar Pradesh, New Delhi and Andhra Pradesh.

4.3.2 The Committee noted that discussion on the application was deferred by the GEAC in its 119th meeting held on 25.4.2014 as experts were of the view that more time is needed to review the biosafety data. It was also decided to forward the biosafety dossier submitted by the applicant on completion of BRL-I trials to all members of the GEAC pursuant to which the proposal would be considered by the Committee.

4.3.3 The Committee noted that the applicant has completed 2 years of BRL-I trials at three locations within their own research farms namely; Jalna, Guntur and Varanasi during 2009-2010.

4.3.4 The Committee also noted that the objectives of the BRL-II trials are to generate:

- Efficacy data of the Bt brinjal against target pest shoot and fruit borer (*Leucinodesorbonalis*).
- Information on yield and demonstrate agronomic performance of Bt brinjal hybrids as compared to their non-Bt counterparts and commercial check hybrids.
- Information on incidence of beneficial and non-target insects among Bt brinjal and their non-Bt counterparts.
- Information on insect infestation on Bt brinjal, their non-bt counter parts and non-bt commercial check hybrids

4.3.5 The Committee took note of the experiment design and proposed isolation measures as given below:

1. Non replicated BRL-II Trials

a. Treatments: 5 nos.

T1	BtJanak hybrid
T2	Non BtJanak hybrid
T3	Bt BSS-793 hybrid
T4	Non Bt BSS-793 hybrid
T5	Non Bt Local Check

b. An isolation distance of 300 m will be maintained.

2. Observations:

A. Entomology:

i. Infield:

- Shoot Damage,
- Larval count
- Fruit Damage
- Stem borer damage
- Non target and beneficial insect pests

B. Yield Parameters

C. Agronomic and Pest Management

i. Economic Threshold Level (ETL)

4.3.6 The Committee observed the following information on the gene construct and transformation method:

Transformation method: Agrobacterium mediated transformation

Detail of Plasmid	Events
The name of the plasmid vector used for transformation	Plasmid Vector: pBinAR
What are the promoter and the terminator used for expressing the gene of interest/s?	Promoter = CaMV35S Terminator = Ocs
The antibiotic resistance marker used in transformation (inclusive of promoter and terminator). Are additional antibiotic resistance markers present in the transgenic plant?	Neomycin phosphotransferase II (npt II) Nos promoter and Nos terminator
Is any reporter gene such as GUS or GFP present in the transgenic plant	No
Is the plant a marker free transgenic?	No

4.3.7 The Committee also took note of the fact that M/s. Bejo Sheetal Seeds Pvt. Ltd., Jalna had made a detailed presentation to the RCGM in its 113th meeting held on 22.05.2012 on the safety and efficacy of the product. RCGM noted that the data submitted by the Company is in order and recommended to the GEAC for BRL-II trials.

4.3.8 The Committee considered the following documents which were circulated to all the expert members on 26.5.2014 to review the data.

A. Application form containing the following information:

- ❖ Introduction
- ❖ Brinjal Crop Biology
- ❖ Molecular Characterization
- ❖ Field Trials Plan
- ❖ Executive Summary. Field Trial Permits
- ❖ BRL-I Field Trial Report of trial during Kharif-2009 and 2010

B. Reports containing results of the following studies:

- a. History of Safe use of *Bacillus thuringiensis*
- b. Safe use of Cry 1 F Protein.
- c. Mode of action of Cry1 Fa1.
- d. Safety assessment of Bt Brinjal carrying *Cry1Fa1 gene*, event 142.
- e. Test with Cry 1 Fa1 purified. Study Center: National Institute of Nutrition,

Hyderabad.

- Pepsin Digestive Assay.
 - Thermo stability.
 - Acute oral toxicity.
- f. Sub-chronic Feeding Test with Bt and non Bt Brinjal leaves and fruits. Study Center: National Institute of Nutrition, Hyderabad.
 - g. Comparative studies on Soil Ecosystem of Bt and Non Bt Brinjal field. Study Center: Institute of Microbial Technology, Chandigarh.
 - h. Detection of CryIFa1 protein in the soils of Bt brinjal trial fields.
 - i. Comparative studies on compositional study of Bt and Non Bt brinjal study Center: Institute for Analysis of Dairy, Food and cultures, Bangalore.
 - j. Pollen flow study.

4.3.9. Two of the Members informed that they have not received the biosafety dossier. Member Secretary GEAC informed that the same would be circulated. Dr B Sesikeran, Member GEAC informed that as former Director NIN, he was involved with the toxicity studies conducted at NIN and therefore he has not forwarded any comments.

4.3.10. During the deliberations and at the request of the Committee, Dr Sesikeran briefly explained the findings of the toxicology studies which are summarized below:

1. The assessment of allergenicity of Bt Brinjal and acute oral toxicity of Cry 1 Fa 1 (event 142) gene has been done as per ICMR guidelines, 2008 and Codex. The protocols for the studies are as per DBT guidelines.
2. The results of pepsin digestibility assay showed that the test protein was rapidly degraded by pepsin in SGF and 90% digestibility was achieved at > 0.5 mins and hence the risk of allergenicity from these proteins in food may be considered to be limited.
3. On the basis of insect bioassay and SDS-PAGE analysis the Cry1Fa1 recombinant protein can be considered heat liable at 95⁰ c when the mortality of target insect of both the test proteins was less than the untreated control test proteins at 10% of target conc.
4. In acute toxicity study with **Swiss Albino Mice** there were 20% mortality reported upon administering of test compound (cry 1 Fa1 lyophilized protein) orally at a dose of 2000 mg/kg body weight in mice. In surviving animals the feed intake, gain in body weight was normal. In addition, there were no significant differences in physical and physiological activities as assessed by various parameters. The histopathological changes observed in the lungs and liver of died animals are common to colony bred animals and may not attribute to administration of test compound. When tests were repeated test again in mice, there was no mortality.
5. In acute toxicity study with **Sprague Dawley Rats**, the test compound (cry 1 Fa1 lyophilized powder) administered orally at a dose of 2000 mg/kg body weight in rats as per regulatory guidelines did not show and mortality and morbidity. The food intake, gain in body weight was not abnormal. In addition, there were no significant differences in physical and physiological activities as assessed by various parameters.
6. In **90 days Rat feeding study** with leaves as well as fruit (2 studies), one female rat from non-transgenic leaf fed group died on day 38. No mortality in transgenic group

was observed. There were no significant differences in body weight gain, organ weights between transgenic and non-transgenic groups. There were no histopathological changes which can be attributed due to the exposure to transgenic / non transgenic test material. However some statically significant differences observed were also within the normal range, and were not found to be biologically significant. Compositional equivalence was established between non transgenic vs transgenic.

4.3.11 Some of the Members were of the view, that the Protocols for 14-days acute oral toxicity study and 90 days sub-chronic toxicity study may need to be revisited as the number of days and sample size of 20 animals (10 male and 10 female) may not be adequate. It was clarified that the animal numbers used in the study are accepted world-wide for pre-clinical or non-clinical evaluation of safety and as per WHO document on safety evaluation of vaccines. Principles of statistics are the same, it is just the test material which is different. It was further stated that the size of the treatment group depends on the animal model chosen, i.e., the number of animals included in studies using non-human primates would be expected to be less than in studies including rodents. For small animal models, e.g., rats and mice, it is recommended that approximately 10 animals/sex/group be studied.

4.3.12 As regards the acceptability of the statistical variations in the toxicity studies even in the absence of any biological significance, Dr Ramesh Sonti supported by three other Members were of the view that *"In acute and sub-chronic toxicity studies, for parameters where there is a statistically significant difference between control and treatment, the test should be repeated for that particular parameter/s (and not for all parameters). If there is a statistically significant difference on repetition, the matter should be investigated further. The matter should be dropped if there is no difference upon repetition. This is being suggested as a matter of 'precautionary principle' because these are foods that will be consumed by a large number of individuals over a long period and we would like to minimize risks. Furthermore, we should go the extra mile to minimize concerns regarding GM foods, at least in the initial years of their commercialization."*

4.3.13 The Committee after a lengthy discussion, decided to constitute a sub-committee to review the toxicity data in the context of the above discussions.

4.3.14 The Committee also noted that IBSC has recommended the proposal in its 20th meeting held on 31.1.2012 and RCGM has recommended BRL-II trials in its 113th meeting held on 22.5.2012

4.3.15 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved BRL-II trials with two transgenic Bt Brinjal hybrids namely Janak and BSS-793 Bt, containing *Cry1Fa1* (Event 142) gene at seven locations namely Maharashtra, Gujarat, Punjab, Haryana, Uttar Pradesh, New Delhi and Andhra Pradesh during any appropriate season subject to the following conditions:

- i. NOC from the State Government where the trials will be conducted.
- ii. Repeat Southern analysis in a single plant copy insertion to demonstration inheritance.

- iii. Toxicity studies to be examined by a sub-Committee.

4.4 Permission to conduct BRL-II trials for transgenic mustard hybrid (DMH-11) (*Brassica juncea*) Events bn 3.6 (Barnase Line), modbs 2.99 (Barstar Line) &bn 3.6xmodbs 2.99 containing *bar*, *barnase* and *barstar* genes by Centre for Genetic Manipulation of Crop Plants, (CGMCP), University of Delhi South Campus, New Delhi.

4.4.1. The Committee considered the request of CGMCP, to conduct Biosafety Research Level-II (BRL-II) trials on transgenic mustard hybrid (DMH-11) (*Brassica juncea*) containing *bar*, *barnase* and *barstar* genes [Events bn 3.6 (Barnase Line), modbs 2.99 (Barstar Line) &bn 3.6xmodbs 2.99]. The trials will be conducted by India Council of Agriculture Research (ICAR) in their respective university's land at ten locations namely; Navgaon, Sriganaganagar, Kumher, Delhi, Bawal, Ludhiana, Bhatinda, (Zone II), Bharatpur, Morena, Kanpur and Faizabad (Zone III). The size of each trial will be 2142 sq m. At the outset, Dr Akshay Pradhan, Member GEAC informed that as a Scientist from CGMCP, he would like to be excused from the deliberations to avoid any conflict of issue unless the committee desires to seek any clarifications.

4.4.2 The Committee noted that the GEAC in its meetings held on 29.9.2010 and 21.09.2011 had approved BRL-I (first year and 2nd year) trial of above mentioned two events bn 3.6 (Barnase Line), modbs 2.99 (Barstar Line) at Bharatpur, Alwar, Sriganaganagar, Kanpur, Ludhiana and Morena.

4.4.3 The Committee also noted the objectives of the trials are to:

- i. Collect data on reproductive and survival biology parameters such as growth, life cycle, plant height, biomass, impact on pollinators etc. of transgenic *Brassica juncea* lines and their non-transgenic counterparts.
- ii. Observe the susceptibility to pests and diseases in transgenic entries as compared to their non-transgenic counterparts.
- iii. Study the impact on beneficial organisms
- iv. Collect data regarding agronomic performance of the hybrid DMH-11 in comparison to national and zonal checks.

4.4.4 The Committee took note of the field experiment design and proposed isolation measures as given below:

- Randomized Block Design with five replications with transgenic and non-transgenic mustard hybrids.
- An isolation of 50 m would be maintained on all sides.
- Transgenic parents namely Varuna barnase (event bn3.6) and EH2 barstar (event modbs 2.99), One non transgenic parent (EH2), one national check (Varuna) and one zonal check would be planted along with the transgenic mustard hybrid DMH-11.

- The anticipated post-trial land use will be restricted for *B. juncea* sowing for next two years and would be studied for volunteers. Any other incompetent crop like wheat etc may be sown.
- All plant material after the harvesting seeds would be burnt in the presence to State Agriculture Department and trial-in-charge.
- Any surplus material such as seeds would be transported back to CGMCP as per the prescribed SOPs.

4.4.5 Committee considered the following information on the gene construct and transformation method:

Transformation method: *Agrobacterium* mediated transformation.

Detailed information regarding genes and vectors:

Detail of Plasmid	Events
The name of the plasmid vector used for transformation	Varunabarnasebn 3.6 – pstbn (pPZP200 backbone) EH2 barstarmodbs 2.99 – pModbs (pPZP200 backbone)
What are the promote and the terminator used for expressing the gene of interest/s?	barnase gene - promoter is TA29 and terminator 35SpA barstar gene - promoter TA29 and terminator 35S pA
The antibiotic resistance marker used in transformation (inclusive of promoter and terminator). Are additional antibiotic resistance markers present in the transgenic plant ?	No antibiotic resistance marker in present in any of the two transgenic lines. <i>Bar</i> gene which confers resistance to herbicide Basta has been used for in vitro selection. Herbicide Basta will also be used to select barnase containing transgenic plants during hybrid seed production. The barnase lines cannot be made homozygous and a Varunabarnase x Varuna (A x B) cross will segregate 1:1 for the barnase gene. Herbicide will have to be used to eliminate the plants without the <i>barnase</i> gene. However, herbicide Basta will only be used for hybrid seed production and not as a general herbicide. Mustard does not have any major weed problems. <i>bar</i> gene in Varunabarnase has 35S promoter and OCS terminator <i>bar</i> gene in EH2 barstar is driven by a 35S double enhancer promoter and has OCS terminator
Is any reporter gene such as	No

GUS or GFP present in the transgenic plant	
Is the plant a marker free transgenic?	No. <i>bar</i> gene has been used as a marker for both in vitro and in vivo selection

4.4.6 The Committee also noted that the CGMCP has completed safety studies as per the prescribed guidelines and the relevant documents have been forwarded to GEAC Expert members. Two of the Members informed that they have not received the biosafety dossier. Member Secretary GEAC informed that the same would be circulated. Committee considered the following documents:

i) Molecular characterization studies:

- a) Description of plant system.
- b) Introduced genes, regulatory sequences and spacer fragment.
- c) Methods of genetic transformation.
- d) Characterization of the transgenic lines.
- e) Genetic stability of the introduced genes.
- f) Target gene efficacy.
- g) Selection of transgenic events and their use in the development of *B. juncea* hybrid DMH-11.
- h) Expression of the introduced genes *bar*, *barnase* and *barstar*.

ii) Safety Assessment Studies-Food safety studies:

- a) Cloning, purification and production of pure proteins.
- b) Bioinformatics analysis of the *Bar*, *barnase* and *Barstar* proteins.
- c) Pepsin digestibility of *Bar*, *Barnase* and *Barstar* proteins.
- d) Thermal stability of *Bar*, *Barnase* and *Barstar* proteins.
- e) Acute oral toxicity of *Bar*, *Barnase* and *Barstar* proteins.
- f) Sub-chronic toxicity studies.
- g) Compositional analysis of key components.

iii) Environmental Safety Assessment Studies:

- a) Studies on weediness potential and aggressiveness parameters: comparative assessment of seed germination and seedling vigour.
- b) Impact on soil microflora.
- c) Crossability and pollen flow studies on the transgenic *B. juncea* hybrid DMH-11: study for intra-specific and inter-specific gene flow.
- d) Studies on pollination behaviors of transgenic *B. juncea* lines: comparative studies on pollen morphology and physiology.
- e) Agronomic performance of hybrid DMH-11 compared to local and national checks.

iv). Detection Protocols:

- a) Protocol for testing at a level of detection (LOD) of 0.01%.
- b) Development of ELISA kits for *Bar*, *Barnase* and *Barstar* .

4.4.7. The Committee observed that the proposal has been recommended by IBSC and RCGM in its meetings on 18.06.2012 and 22.4.2014 respectively with the conditions (i) that during the BRL-2 trials an entomologist should be included; (ii) data should be recorded on beneficial insects; no. of pollinators per plant or per meter row; and (iii) no. of replications should be increased to 5.

4.4.8 The Committee discussed the application in detail and appreciated the molecular analysis which has been carried and compilation of the biosafety dossier. The Committee noted the following information with respect to the 3 genes present in the transgenic mustard DMH-11:

- i. The *Barnase gene* (333bp) has been derived from bacterium *Bacillus amyloliquefaciens*, commonly occurring soil bacterium encoding a ribonuclease called Barnase, which degrades RNA in the tapetum at early stages of pollen formation. The eventual complete loss of RNA in the restricted cell layer leads to the death of these cells expressing the ribonuclease enzyme. In turn, this leads to the deposition of wound callose, which prevents nutrients reaching the tissues of the anther filament, thereby leading to wilting of the anther. Consequently, plants containing the *barnase gene* are phenotypically normal except that, during flowering, the shape of the anther is altered and pollen production is reduced.
- ii. The *Barstar gene* (273bp) is also derived from *Bacillus amyloliquefaciens* phenotypically the same organism from which the barnase gene was isolated. The barstar gene encodes Barstar protein which inhabits the RNase activity of Barnase by forming a one to one complex with the Barnase protein. A codon modified sequence of the barstar was used for the development of restorer barstar lines so as to express the Barstar protein at a higher level for efficient fertility restoration of the barnase gene containing male sterile lines.
- iii. The bar gene (552bp) has been derived from *Streptomyces hygroscopicus* and encodes for enzyme phosphinothricin acetyl transferase (PAT). PAT detoxifies the compound DL-Phosphinothricin (PAT), an active compound of the herbicide glufosinate ammonium by acetylation. *Streptomyces spp.* are saprophytic, soil borne microbes and are not considered to be a pathogen of plants, humans, or other animals.

4.4.9 Dr Sonti informed that the Barnase Protein is fundamentally a toxic protein and used as an anti-cancer drug. It was clarified that the protein is expressed only in the tapetum and is not expressed in any other tissue as per the analysis carried out in the report. Clarifications were also sought on why very low level of expression barstar is found in other tissues in addition to anthers but not barnase even though both the genes are expressed under the same tapetum specific promoter. At the request of the Committee, Dr Pradhan clarified that in the barstar construct the selection marker bar gene is driven by 35S double enhancer promoter (a stronger promoter than the normal 35S promoter) and the barstar gene by tapetum specific TA29 promoter. In the barnase construct the bar gene is driven by normal 35S promoter and barnase gene by TA29. The barstar expression observed in tissues other than the anthers could be due to the enhancer element of 35S double

enhancer promoter influencing the TA29 promoter and thus resulting in very low expression of barstar in tissues other than anthers.

4.4.10 On the issue of the toxicology studies consensus was expressed that unlike in the west, GM Canola is used as oil whereas in India mustard leaves and seeds are also consumed and therefore, toxicology data should be reviewed with great caution. The Committee decided to refer the matter to the sub-committee proposed under agenda item no 4.3.

4.4.11 In addition, it was decided to obtain the following additional information:

- What are the other hybrids of Canola approved for commercial cultivation in the West and how much of the cultivated hybrids fall under barnase and barstar genes.
- Whether Barnase is expressed in other tissues
- Whether Barstar is expressed in other tissues.

4.4.12 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved BRL-II trials for transgenic mustard hybrid (DMH-11) (*Brassica juncea*) Events bn 3.6 (Barnase Line), modbs 2.99 (Barstar Line) & bn 3.6 x modbs 2.99 containing *bar*, *barnase* and *barstar* genes at Navgaon, Sriganaganagar, Kumher, Delhi, Bawal, Ludhiana, Bhatinda, Bharatpur, Morena, Kanpur and Faizabad during any appropriate season subject to submission of NOC from the State Government. The Committee further decided to obtain additional information as indicated in para 4.4.11 above and also decided to refer the matter to the sub-committee proposed for the purpose of review to the toxicology data under agenda item 4.3.13 and 4.4.10.

4.5 Permission for conduct of experimental seed production of transgenic *B. juncea* EH2 barstar and non-transgenic EH2 line for livestock feeding studies by Centre for Genetic Manipulation of Crop Plants, University of Delhi South Campus, New Delhi.

&

4.6 Permission for conduct of confined field trials for the experimental leaves production of transgenic *B. juncea*EH2 barstar and non-transgenic EH2 line for live stock feeding studies at NDRI, by Centre for Genetic Manipulation of Crop Plants, University of Delhi South Campus, New Delhi.

4.5.1 The Committee considered the above request of CGMCP, New Delhi to conduct experimental seed and leaf production of transgenic *B. juncea*EH2 barstar and non-transgenic EH2 lines for livestock feeding studies, at one location in the farm land owned by University of Delhi at Jaunti Village, in an area of 2 acres (8094 sq meter). The seed meal will be transferred to NDRI, Karnal by CGMCP for livestock feeding studies with cattle.

4.5.2 The Member Secretary, RCGM informed that the applicant had sought exemption from feeding studies with leaves and seeds as they are not toxicological studies but basically meant to evaluate nutritional imbalance. As compositional equivalence of edible plant parts (leaf and seeds) has been established, and no Allergenicity has been observed, additional feeding studies may not be required. Further, the same genetic system has been in use for more than 15 years in several countries around the World. The matter was considered by the RCGM in its 133rd meeting held on 22.4.2014. While the RCGM agreed to the above, the Secretariat was advised to send a brief note to the GEAC with justification on why feeding studies are not required in the present case.

4.5.3 After a brief discussion on the matter, the Committee requested that the Note forwarded by RCGM may be circulated to all members of the GEAC for consideration of the case in the next meeting. Accordingly decision on the two proposals mentioned above were deferred.

4.7 Permission to conduct event selection trials of transgenic rice (*Oryza sativa*) events namely; OSLR-01 and OSLR-04 containing *cry1Ab* (DG) gene by M/s. Devgen Seeds & Crop Technology Pvt. Ltd., Secunderabad

4.7.1 The Committee considered the request of M/s. Devgen Seeds & Crop Technology Pvt. Ltd., Secunderabad to conduct event selection trials of transgenic rice (*Oryza sativa*) events namely; OSLR-01 and OSLR-04 containing *cry1Ab* (DG) gene for resistance to feeding damage incurred by Lepidopteron pests. The trials will be conducted at one trial per location in an area of 678 m² at State Agricultural Universities; Company owned sites or long term leased land. Details of location will be provided after obtaining NOC from State Governments.

4.7.2 The Committee noted that Devgen has generated 2 events of *Cry 1A* gene in tropical Japonica and Indica rice. Event evaluation will be done with 2 events in three different genotypes, with 6 GM test treatments and 3 isogenic non-GM genotypes.

4.7.3 The Committee noted the objective of the trials is to:

- Compare morphological traits of transgenic rice with non-transgenic counterpart including grain yield and quality.
- Compare other characteristics of transgenic and non-transgenic rice hybrids such as susceptibility towards other pests and disease, and impact on non-target organisms.

4.7.4 The Committee also took note of the field experiment design and proposed isolation measures as given below:

- Spatial isolation distance with guard rows.
- Five meters of non-transgenic refuge rice crop will be planted at the border region around the experimental field.

- Distance to nearest cultivated crop of the same species will be at least 200 m (isolation).
- The trial site will be secured by a fence
- Split plot, with Chassis as whole plot arranged in four replications of randomized complete blocks and event as split plot treatment. At each location and within each replicate, chassis order is randomized, then event randomized within chassis.

4.7.5 The Committee considered the following information on the gene construct and transformation method:

Transformation method: Agrobacterium mediated transformation.

Detailed information regarding genes and vectors:

Detail of Plasmid	Events
The name of the plasmid vector used for transformation	<i>A. tumefaciens</i> co transformation- pGCF027 & pGCF055
What are the promoter and the terminator used for expressing the gene of interest/s?	promoter - <i>Double-enhanced CaMV 35S</i> terminator - <i>CaMV 35S</i>
The antibiotic resistance marker used in transformation (inclusive of promoter and terminator).	Double enhanced CaMV 35S promoter, <i>nptII</i> coding sequence, <i>nos</i> Transcription Terminator. However this antibiotic resistance marker is segregated out, so is not present in this material.
Are additional antibiotic resistance markers present in the transgenic plant?	No
Is any reporter gene such as GUS or GFP present in the transgenic plant	No
Is the plant a marker free transgenic?	Yes

4.7.6 The Committee observed that the proposal has been recommended by IBSC RCGM in its meeting held on 24.10.2013 and 25.02.2014 respectively.

4.7.7 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved conduct of event selection trials of transgenic rice (*Oryza sativa*) events namely; OSLR-01 and OSLR-04 containing *cry1Ab* (*DG*) gene during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted. The applicant was also advised to submit information on (i) Source of gene, (ii) Locations where trials are to be conducted and (iii) the target pest

4.8 Request for inclusion of two additional locations viz., Abohar (Punjab) and Gandhi Nagar (Gujarat) for the conduct of event selection trial on transgenic rice (*Oryza sativa*) events MHRM01 to MHRM20 containing *cry1Ab* & *cry1C* genes by M/s. Metahelix Life Sciences Limited, Bangalore.

4.8.1 The Committee noted that the GEAC in its 120th meeting held on 12.5.2014 had accorded approval to conduct event selection on transgenic rice (*Oryza sativa*) marker free Bt events namely MHRM01 to MHRM20 containing *cry1Ab* gene for resistance to Rice Yellow Stem Borer (*Scirpophagaincertulas*) at Vattinagulapalli Village, RR Dist. Andhra Pradesh during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.8.2 The Committee considered the present request of the company to include two more locations i.e. Abohar (Punjab) and Gandhi Nagar (Gujarat) in addition to Vattinagulapalli village, Ranga Reddy District, Telangana. The applicant has further informed that the proposed trials at the two additional locations are exactly the same as the one for which application was made previously i.e. with the same events, genes, trial plans etc

4.8.3 The Committee noted that the RCGM has recommended the request in its 132nd meeting held on 25.03.2014.

4.8.4 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for inclusion of two additional locations viz., Abohar (Punjab) and Gandhi Nagar (Gujarat) for conducting event selection trial on transgenic rice (*Oryza sativa*) events MHRM01 to MHRM20 containing *cry1Ab* & *cry1C* genes during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.9 Permission to conduct Biosafety Research Level-1 (BRL-1) trial on brinjal (*Solanummelongena* Linn.) hybrids namely; Ajay Bt 1, Vijay Bt 1 and KirtiBt 1 containing *Cry1Fa1* gene (ANK-19 event) by M/s. Ankur Seeds Pvt. Ltd., Nagpur

4.9.1 The Committee considered the request of M/s. Ankur Seeds Pvt. Ltd., Nagpur for to conduct Biosafety Research Level-1 (BRL-1) trial on brinjal (*Solanummelongena* Linn.) hybrids namely Ajay Bt 1, Vijay Bt 1 and KirtiBt 1 containing *Cry1Fa1* gene (ANK-19 event) stably integrated into the brinjal genome and imparting resistance to lepidopteran pests, particularly to *Leucinodesorbonalis*Guen. and *Spodopteralitura* (F.) The trial is proposed to be conducted at 16 locations namely:

- (i) Punjab Agricultural University (PAU), Ludhiana (Punjab)
- (ii) CCS-Haryana Agricultural University (CCSHAU), Hisar (Haryana)
- (iii) Indian Institute of Vegetable Research (IIVR), Varanasi (Uttar Pradesh)
- (iv) Ankur Research Farm, Village:Rupal, Dist.: Gandhinagar (Gujarat)
- (v) Junagadh Agricultural University (JAU), Junagadh (Gujarat)
- (vi) Anand Agricultural University (AAU), Anand (Gujarat)
- (vii) Ankur Research Farm, Village: Kinhi, Dist.: Nagpur (Maharashtra)
- (viii) Dr. PunjabraoDeshmukhKrishiVidyapeeth (Dr. PDKV), Akola (Maharashtra)

- (ix) Mahatma PhuleKrishiVidyapeeth (MPKV), Rahuri, Dist.: Ahmednagar (Maharashtra)
- (x) Odisha University of Agriculture and Technology (OUAT), Bhubaneswar (Odisha)
- (xi) Ankur Research Farm, Geedipally (Andhra Pradesh)
- (xii) Dr. YSR Horticultural University (Dr. YSRHU), West Godavari Dist (Andhra Pradesh)
- (xiii) Acharya N.G. Ranga Agricultural University (ANGRAU), Hyderabad (Andhra Pradesh)
- (xiv) International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru (Andhra Pradesh),
- (xv) Indian Institute of Horticultural Research (IIHR), Bangalore (Karnataka) and
- (xvi) Tamil Nadu Agricultural University (TNAU), Coimbatore (Tamil Nadu).

4.9.2 The Committee noted that the trial site locations will be finalized after receiving consent from the concerned Institute/SAU for conducting the trial in an area of 2295.7 sqm/location. The final list of the trial site locations will be submitted along with the NOC from the respective State Government.

4.9.3 The Committee noted the objectives of the trials are to:

- Evaluate three transgenic brinjal (*S. melongena*) hybrids with ANK-19 event. Evaluation will be done based on the resistance/protection offered against the *Lepidoteran* pests particularly to *Leucinodesorbonalis* (Guen.) and *Spodopteralitura* (F).
- Confirm consistency of Cry1Fa1 protein expression across the growing season.
- Examine the morphological equivalence with the non-transgenic counterpart.

4.9.4 The Committee also took note of the field experiment and proposed isolation measures method as given under:

- Trial will be conducted in Randomised Block Design (RBD) .
- 300 meter isolation distance will be maintained.
- The trial plot will be surrounded by non-transgenic brinjal crop as buffer zone.

4.9.5 The Committee considered the following information on the gene construct and transformation method:

- (i) Plasmid description: pGCF027
- (ii) Transformation method: Agrobacterium mediated transformation

4.9.6 The Committee observed that the proposal was earlier considered by RCGM in its 119th meeting held on 26.12.2012 and requested the applicant to submit the following information:

- Original genotypes of the crop which is transformed.

- Information on the event specific primer sequence.
- Ambiguity regarding the gene which is used, as the applicant has used different terminology in the application i.e cry1F, cry1Fa and cry1Fa1 gene.
- Information on the southern analysis of the proposed gene
- The vector containing marker gene (nptII) has been used during the transformation, however in the plasmid map of the plant transformation vector (pBin-F) marker gene is not shown.
- Information/ objectives regarding the biosafety data to be generated

4.9.7 The Committee also observed that the proposal has been recommended by IBSC and RCGM in its meetings held on 18.10.2012 and 25.03.2014.

4.9.8 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for conduct of BRL-1 trial on brinjal (*Solanum melongena* Linn.) hybrids namely Ajay Bt 1, Vijay Bt 1 and Kirti Bt 1 containing *Cry1Fa1* gene (ANK-19 event) 16 locations at namely; Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra, Odisha, Andhra Pradesh, Karnataka and Tamil Nadu during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted. The applicant is also advised to submit details of source of gene.

4.10 Request for inclusion of an additional location for conduct of event selection trial of Water Use Efficient (WUE) rice containing *ipt* gene in the State Agriculture University Farm in Maharashtra by M/s. Maharashtra Hybrid Seeds Company Limited (MAHYCO).

4.10.1 The Committee noted that the GEAC in its 119th meeting held on 25.4.2014 had accorded approval to conduct event selection on Water Use Efficient (WUE) rice containing *ipt* gene at *Anand Nagar, Nizamabad district (Andhra Pradesh)* during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.10.2 The Committee took note that the Company has not received NOC from Andhra Pradesh till today. The present request of the company is to include two more locations at State Agriculture University Farm at Mahatma Phule Krishi Vidyapeeth, Rahuri or Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli in Maharashtra in addition to Telangana.

4.10.3 The Committee observed that the request has been recommended by RCGM in its meeting held on 26.05.2014.

4.10.4 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for inclusion of an additional locations for conduct of event selection trial of Water Use Efficient (WUE) rice containing *ipt* gene at State Agriculture University Farm at Mahatma Phule Krishi Vidyapeeth, Rahuri or Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (DBSKKV) Dapoli in

Maharashtra in addition to Telangana during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.11 Request for change of location for conduct of event selection trial of Nitrogen Use Efficient (NUE) rice containing *AlaAt* gene by M/s. Maharashtra Hybrid Seeds Company Limited (MAHYCO).

4.11.1 The Committee noted that the GEAC in its 115th meeting held on 8.2.2012 had accorded approval to conduct event selection trials with transgenic rice (*Oryza sativa* L.) events; namely MW-01 to MW-25 containing the *AlaAt* gene at company's own research farm at Anand Nagar, Nizamabad district (Andhra Pradesh/ Telangana) during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.11.2 The Committee also observed that GEAC in its 118th meeting held on 21.3.2014 has changed the location from AP to Maharashtra, extended validity period and protocol to conduct trials at SAU's research farm at DBSKKV, Panvel, during any appropriate season.

4.11.3 The Committee noted that the present request of the Company is to further change the location of the trial site from Panvel to DBSKKV, Dapoli, Maharashtra during appropriate season due to non-availability of land to conduct trials at Panvel,

4.11.4 The Committee observed that the request has been recommended by the RCGM in its meeting held on 26.5.2014

4.11.5 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for change of location for conducting event selection trial of Nitrogen Use Efficient (NUE) rice containing *AlaAt* gene at Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, (DBSKKV) Dapoli, during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted

4.12 Request for change of location for conduct of event selection on BtBrinjal *Cry1Fa1* gene from Attur, Tamil Nadu to Gurgaon, Haryana by M/s. Rasi Seeds(P) Ltd., Tamil Nadu

4.12.1 The Committee noted that the GEAC in its 120th meeting held on 12.5.2014 had accorded approval for conduct of event selection trials on Bt Brinjal *Cry1Fa1* gene at R & D Centre, Attur, Tamil Nadu during the appropriate season subject to submission of NOC from the State Department of Agriculture where the trials would be conducted.

4.12.2 The Committee noted that the present request of the applicant is to change the trial site from Attur, Tamil Nadu to Gurgaon, Haryana in their own R&D farm procured for vegetable breeding.

4.12.3 The Committee observed that the request has been recommended by RCGM in its meeting held on 26.5.2014

4.12.4 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for change of location for conduct of event selection trials on Bt Brinjal *Cry1Fa1* gene from Attur, Tamil Nadu to Gurgaon, Haryana, during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.13 Request for additional location for conducting event selection trials (EST15, EST16 and EST17) on SPT transgenic rice (*Oryza sativa L.*) *DsRed2*, *Zm-AA1*, *OsMSCA1* genes submitted by M/s. Pioneer Overseas corporation India Pvt. Ltd., Hyderabad.

4.13.1 The Committee noted that the GEAC in its 120th meeting held on 12.5.2014 had approved the following three applications for conduct of event selection trials on SPT transgenic rice (*Oryza sativa L.*) containing *DsRed2*, *Zm-AA1*, *OsMSCA1* genes during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted:

- Event selection trial (EST15) on 10 SPT1 and 10 SPT6 Rice (*Oryzasativa L.*) at any two location in Medak Distt and Nizamabad distt Telangana , and Navsari and Anand Agricultural. University in Gujarat.
- Event selection trial (EST16) on 10 SPT1 and 10 SPT6 Rice (*Oryzasativa L.*) at 2 locations, in Andhra Pradesh (Masaipet Village, Medak Distt , and in Gujarat (Anand Agricultural University, Navasari Agricultural University)
- Event selection trial (EST17) on 16 SPT1 and 16 SPT6 Rice (*Oryzasativa L.*) at one location each in Medak Distt and Nizamabad distt Telangana, and Navsari and Anand Agricultural University in Gujarat.

4.13.2 The Committee considered the present request of the Company for inclusion of following two additional testing locations in Rajasthan:

- ✓ Maharana Pratap University of Agriculture & Technology, Udaipur.
- ✓ Kota Agricultural University, Kota

4.13.3 The Committee observed that the request has been recommended by RCGM in its meeting held on 26.5.2014.

4.13.4 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request for additional location for conducting event selection trials (EST15, EST16 and EST17) on SPT transgenic rice (*Oryza sativa L.*) *DsRed2*, *Zm-AA1*, *OsMSCA1* genes during any appropriate season subject to submission of NOC from the State Government where the trials will be conducted.

4.14 Permission for F1 Hybrid seed production (Two cycles / year) from constructs RPD5-RPD17 two at locations each in North, Central, South and East Zones by M/s. BASF India Ltd, New Delhi.

4.14.1 The Committee noted that the GEAC in its 118th meeting held on 21.3.2014 had considered the request of the company for F1 Hybrid seed production (Two cycles / year) from constructs RPD5-RPD17 at two locations each in North, Central, South and East Zones. The decision on the request was deferred as the Committee was of the view that only limited seed production for conducting additional field trials may be allowed. It was therefore decided to advise the applicant to submit minimum seed production required for future trials with due justification on the quantity as well as area required.

4.14.2 The Committee considered the following information /justification submitted by the applicant vide their letter No BASF/GEAC/05/2014-(103) dated 5.5.2014:

"The F1 seed production is an essential step to produce test hybrid seed with 1-3 female non-transgenic cytoplasmic male sterile lines and a transgenic event. The produced test hybrids will be tested in Elite event Selection Field trials in different agro-climatic ecosystems and finally the appropriate Elite Event will be selected. Production takes place in the open field: The Transgenic male line (Event) is planted at different planting dates with the non-transgenic female lines in order to assure a synchronized flowering of male and females. Only the seed harvested on the female lines will be used for yield trials. The seed of the male lines will be destroyed. The average area required for this purpose is 1000m² (0.01 ha). The quantity of seed produced in one location would be approximately 400 grams per event and female cross, resulting in a total with 168 events 3 females of 504 F1 hybrid crosses and approximately 400 grams seed per cross. The amount of seeds produced would be sufficient to run the needed event selection trials in different locations as approved by GEAC.*

Although the primary F1 hybrid seed production for the field trial will happen only at one location we request the committee to give permission in a minimum of one location in any of the two zones considered in the last GEAC communication. .Given the uncertainty that surrounds NOC's this would give us the option to apply for a NOC to different states. The F1 hybrid seed production would however take place in only any one of the approved locations.

For BASF to conduct Event Selection Trial it is imperative to produce the seed this season and hence we kindly request GEAC to approve F1 hybrid seed production with 168 events of transgenic rice constructs RPD5-RPD17, in an area of 1000m² (0.10ha) in one location each in any two zones (Central and South)".

4.14.3 In view of the above stated facts, the Committee approved the request for F1 Hybrid seed production (Two cycles / year) from constructs RPD5-RPD17 at one location each in any two zones during any appropriate season subject to submission of NOC from the respective State Governments where the trials will be conducted. The Committee further directed the applicant not to conduct the event selection trial in proximity to Basmati growing area.

4.15 Permission to conduct event selection trials on transgenic potato with *RB gene* at Central Potato Research Institute (CPRI), campus, Shimla.

4.15.1 The Committee noted that the GEAC in its 111th meeting held on 6.7.2011 had accorded approval for conduct of event selection trails on transgenic potato events namely; SP 951, SP 904, KB/SP951, KB/SP904, KJ/SP951, KBRB, KJRB containing RB gene

4.15.2 The Committee considered the present request of Central Potato Research Institute (CPRI), Shimla to allow them to conduct event selection trials on transgenic potato namely; KJ 16, KJ 21, KJ65, KJ 66 and KJ 77 (Progeny of event KJ/SP951) containing RB gene. It was noted that CPRI has informed that from data already generated under controlled environment chamber, they have selected five RB transgenic hybrids of the popular potato cultivar KufriJyoti, i.e. KJ16, KJ 21, KJ 65, KJ 66, and KJ 77 for BRL-I trial.

4.15.3 The Committee noted that NOC obtained from Himachal Pradesh Govt., covered all different potato genotypes in which RB gene is incorporated. It covered direct transformants as well as RB-hybrids of two cultivars.

4.15.4 The Committee also noted that the RCGM has requested GEAC vide their letters dated 14.5.2012, 22.4.2013 and 5.5.2014, to clarify whether justification provided by the CPRI, Shimla can be agreed and a permit letter can be issued to them for conducting event selection trials on five RB transgenic hybrids of the popular potato cultivar KufriJyoti namely; KJ 16, KJ 21, KJ65, KJ 66 and KJ 77 belonging to KJ/SP951 which contains RB gene.

4.15.5 In view of the above stated facts, the Committee approved the request for conduct of event selection trials on transgenic potato hybrids of the popular potato cultivar KufriJyoti namely; KJ 16, KJ 21, KJ65, KJ 66 and KJ 77 belonging to KJ/SP951 event with *RB gene* at Central Potato Research Institute (CPRI), campus, during any appropriate season subject to submission of NOC from the respective State Governments where the trials will be conducted

Agenda Item No 5: Policy issues

5.1 Request for guidance on safety assessment studies required for stacked event of transgenic corn of Bt11 and GA21 events (stack of Insect tolerant and herbicide tolerant events) submitted by M/s. Syngenta Biosciences Pvt. Ltd., New Delhi.

5.1.1 The Committee considered the request of M/s. Syngenta Biosciences Pvt. Ltd., New Delhi for guidance on safety assessment studies required for stacked event of transgenic corn which was produced by conventional crossing of two single events of Bt11 and GA21. The main issue in the instant case is whether biosafety data generated on both the single events are adequate or biosafety data is required to be generated for the stacked event.

5.1.2 The Committee took note of the following information:

- i. Maize with stacked event of Bt11xGA21 only will be sold as commercial product.

- ii. Single event of Bt11 and GA21 will be released into the environment for commercial F1 seed production of the stack event of maize.
- iii. Both the single events and stack event is approved in a number of countries for food/feed/cultivation.
- iv. Biosafety studies as required by RCGM on both single events of Bt11 and GA21 have been completed and submitted to RCGM. Both the events of stack are approved in many countries and extensive safety data is available.
- v. RCGM in its meeting held on 26.3.2013 and allowed *for conducting the feeding studies on both single events of transgenic corn i.e. Bt11 and GA21 only.*

5.1.3 The Committee also noted that M/s Syngenta has informed that there is no information or experience to indicate that the combination of unrelated traits such as insect resistance and herbicide tolerance would raise any new food, feed or environmental safety issues because each of the genes function independently

5.1.4 Members discussed the need for generation of biosafety data on stacked products if both products have been independently shown to be “as safe as” other commercial crop varieties and whether the combination of the two traits by conventional breeding techniques would raise any new safety concerns as this process is similar to combining conventional traits using traditional plant breeding techniques. Members were of the view that if both the events are in the same genetic background, then repeating the safety assessment may not be required.

5.1.5 The Committee after detailed deliberations decided to obtain information on (i) genetic background (ii) compositional analysis (iii) agronomic performance; (iii) substantial equivalence. Accordingly decision on the proposal was deferred.

5.2 Protocols (two) for conducting feeding studies on rat and broiler chicken using transgenic corn grains at International Institute for Biotechnology and Toxicology (IIBAT), Padappai, Chennai by M/s Syngenta Biosciences Pvt. Ltd., Pune.

5.2.1 The Committee considered the request of M/s Syngenta Biosciences Pvt. Ltd., Pune for approval of two protocols (i) Sub chronic 90 day feeding study in Wistar rats and (ii) Feeding study in broiler chicken at International Institute for Biotechnology and Toxicology (IIBAT), Padappai, Chennai.

5.2.2 As the above request is linked to the decision under agenda item 5.1, it was decided to consider the matter after taking a view on the previous agenda in the next GEAC meeting.

Agenda Item No 6: Application related to Pharmaceuticals:

6.1 Permission for import of Vector Mune Fowl Pox–Mycoplasma Gallisepticum (MG) Poultry Vaccine from USA and Marketing in India by M/s Ceva India Pvt Ltd., Delhi.

6.1.1 The Committee decided in the first instance to obtain comments from the experts prior to placing the proposal in GEAC agenda. Accordingly decision on proposal was deferred.

6.2 Request for revalidation of GEAC permission for manufacture and marketing of Foot and Mouth disease (FMD) Vaccine by M/s Intervet India Pvt. Ltd, Pune.

6.2.1 The Committee considered the request of M/s Intervet India Pvt. Ltd for revalidation of GEAC permission dated 1.6.2009. It was noted that the above request was considered by the GEAC in its meeting held on 21. 3.2014, wherein the Committee opined that in the first instance, the applicant may be advised to submit information on (i) Post Marketing Surveillance data, (ii) Marketing feedback and (iii) Incidence of disease.

6.2.2 The Committee noted that M/s Intervet India Pvt Ltd vide their letter No IIP/RA/GEAC/FMDV/2014/36 dated 14.05.2014 has submitted the following clarifications:

- i. For the last 10 years, FMD vaccine is being manufactured and supplied to Department of Animal Husbandry and Dairying under the Ministry of Agriculture as per tender conditions set on yearly basis.
- ii. Number of FMD vaccines supplied to the Govt from 2009-2013 are 99.66 million doses.
- iii. Number of FMD vaccines supplied to commercial dairy farms from 2009-2013 are 1.26 million doses.
- iv. Post marketing surveillance data is maintained by Department of Animal Husbandry, Delhi. Post-vaccination sero-surveillance amongst few dairy farms do indicates that protective antibody titers are obtained in the farm conditions.
- vi. Regarding marketing feedback it was noted that Department of Animal Husbandry, Delhi is the main customer for FMD vaccine and vaccine is used by them. Same dairy farmer is using the vaccine repeatedly on annual basis with satisfactory results. Technical advice is extended to all dairy farmers through specialized cell called" Veterinary Services Department" as and when required.
- vii. Regarding the Incidence of disease it was noted that FMD is part of control program by Government of India and therefore the disease situation is monitored by the government directly. Sporadic outbreaks are reported whenever unrestricted animal movement is there in few commercial dairy farms. Overall disease situation is under control in dairy farms where strict bio-containment practices are followed which is mandatory for FMD control.

6.2.3 After detailed deliberations, and taking in to the consideration the information provided by the applicant, the Committee decided to revalidate the GEAC permission for manufacture and marketing of FMD Vaccine.

6.3 Permission to conduct Phase III clinical trials on study titled “Immunogenicity and Safety of a Tetravalent Dengue Vaccine manufactured by Sanofi Pasteur, SA Lyon, France in healthy subjects aged 18 to 45 years in India (Protocol No. CYD 48) by M/s Sanofi Pasteur India Private Limited, Mumbai.

6.3.1 The Committee decided in the first instance to obtain comments from the experts prior to placing the proposal in GEAC agenda. Accordingly decision on proposal was deferred.

Agenda Item No 7: Applications related to import of Soybean Oil (reconsidered case)

7.1 Permission to import transgenic Soybean Oil by three company’s viz. M/s. Bayer BioSciences Pvt. Ltd, Gurgaon, M/s by BASF India Ltd and M/s Monsanto Holdings Pvt. Ltd

7.1.1 The Committee noted that the requests of the M/s. Bayer BioSciences Pvt. Ltd, Gurgaon, M/s by BASF India Ltd and M/s Monsanto Holdings Pvt. Ltd, for import of Soybean oil was considered by the GEAC in its 118th meeting held on 23.3.2014 wherein the Committee observed certain discrepancies and decided to obtain clarification from CFTRI on (i) why +ve controls show –ve results and (ii) why – ve controls show + results. Further, the Committee was also of the view that +ve control should be same oil spiked with r-DNA or Protein.

7.1.2 The Committee considered the clarification received from CFTRI and noted that the Test Reports received from CFTRI indicate (a) DNA was absent in Refined Soybean oil and Crude Oil LL Soybean for all events (LL event AA547-127, LL event A2704-12, RR event (BtRR2Y) and event BPS-CV127-9); and (b) No protein was detected by amino acid analysis for all Soybean events mentioned above. The Committee also noted that the tests have been conducted at a detection level of 0.01 %. Further, the test samples have been spiked with nanogram levels of r-DNA for confirming the validity of the tests.

7.1.3 After detailed deliberations, the Committee was in a view that the clarifications provided by the CFTRI are in order and decided to approve the import of Refined Soybean Oil derived from transgenic Soybean by three company’s viz. M/s. Bayer Bio-Sciences Pvt. Ltd, Gurgaon, M/s by BASF India Ltd and M/s Monsanto Holdings Pvt. Ltd.

Agenda Item No 8: Information Item

8.1 Inclusion of Telangana State in the permission letter approved for Confined field trials (CFT) in Andhra Pradesh.

8.1.1 The Committee noted that M/s Monsanto and M/s Pioneer have requested for inclusion of Telangana State in the permission letter approved for confined field trials instead of Andhra Pradesh as the proposed locations are now part of the newly formed state of Telangana and accordingly revised communications have been issued in respect of the following proposals:

- BRL-1 trials on transgenic maize (*Zea mays L.*) expressing MON NK 603 Andhra Pradesh, Gujarat and Rajasthan by M/s Monsanto.
- BRL-II trials and seed production of transgenic maize (Event MON 89034 x NK603) Haryana, Punjab, Bihar, Maharashtra, Tamil Nadu, Karnataka, Andhra Pradesh, Rajasthan and Uttar Pradesh in Kharif season and Andhra Pradesh, Bihar, Karnataka, Maharashtra, Gujarat and Tamil Nadu (six locations) by M/s Monsanto.
- Event selection (Trial-4) on 48 transgenic rice (*Oryzasativa*) Andhra Pradesh/ Gujarat, Maharashtra/Tamil Nadu by M/s Pioneer.

8.1.2 The Committee advised that all future requests may be addressed in the same manner.

Agenda Item No 9: Any other item with the permission of Chair.

Next meeting of the GEAC is tentatively scheduled on 28.8.2014

Annex 1

List of the Members who attended the 121st GEAC meeting held on 18.07.2014

S.No.	Name and address
1.	Shri Hem Pande , Additional Secretary, MoEF& CC and Chairman, GEAC.
2.	Dr K. Veluthambi, Professor (retd) & Head, School of Biotechnology, Madurai Kamraj University, Madurai and Co-chairman, GEAC .
3.	Dr. B. Sesikeran, Former Director, National Institute of Nutrition, Hyderabad.
4.	Dr. Swapan Kumar Datta, DDG (Crop Science), Indian Council of Agricultural Research, Krishi Bhawan, New Delhi.
5.	Dr. Ramesh Sonti, Chief Scientist, CSIR, Centre for cellular & Molecular Biology (CSI-CCMB) Uppal Road, Hyderabad
6.	Prof. Akshay Kumar Pradhan, Department of Genetics, University of Delhi, South Campus, Benito Juarez Road, New Delhi
7.	Dr. V V Ramamurthy, Principal Scientist, Entomology Division, IARI, New Delhi ,Centre for Ecological Sciences, Indian Institute of Science, Bangalore
8.	Dr. Renee M Borges, Professor, Centre for Ecological Sciences, Indian Institute of Science, Bangalore.
9.	Dr. Luther Rangreji, Associate Professor, Faculty of Legal Studies, South Asian University,233, Akbar Bhavan, Chankyapuri
10.	Shri R.K. Mishra, ADC (Seeds) Department of Agriculture & Cooperation Ministry of Agriculture, Krishi Bhawan, New Delhi
11.	Dr. S.S. Banga, Plant Breeder, Punjab Agriculture University, Ludhiana
12.	Shri. R. Murali, Dy. Director (E), Plant Protection Advisor, Directorate of Plant Protection, Quarantine & Storage NH IV, Faridabad-121001
13.	Dr. Meenakshi Singh, Scientist F, Food Safety and Standards Authority of India (FSSAI),FDA Bhawan, Next to Rashtriya Bal Bhavan, Kotla Road, New Delhi
14.	Dr. Ranjini Warriar, Director, Ministry of Environment & Forests and Member Secretary, GEAC.
15.	Smt. Madhu Gupta, Research Officer , MoEF
Special Invites	
16.	Dr. P. M. Bhargava, Former Director ,CCMB, Hyderabad