Decision taken in the 103rd meeting of the Genetic Engineering Appraisal Committee (GEAC) held on 29.9.2010.

The 103nd meeting of the GEAC was held on 29.09.2010 in the Ministry of Environment and Forests (MoEF) under the chairmanship of Shri M.F. Farooqui, Additional Secretary, MoEF and Chairman, GEAC.

The deliberations/decisions taken in the GEAC meeting in respect of Agenda items 4 to 6 are as follows:

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

4.1 Permission to conduct event selection trials on transgenic rice (*Oryza sativa*) events namely MHR01 to MHR566 containing *cry1Ac* and *cry1Ab* gene by M/s. Metahelix Life Sciences Pvt. Ltd., Bangalore.

4.1.1 The Committee considered the request from M/s. Metahelix Life Sciences Pvt. Ltd., Bangalore to conduct event selection trials on transgenic rice events namely MHR01 to MHR566 containing *cry1Ac* and *cry1Ab* genes. The event selection trials will be conducted at one location in an area of 975 sq m within the institutional research farm at Vattinagulapalli village, Ranga Reddy Dist, Andhra Pradesh during July 2010.

4.1.2 The Committee noted that the purpose of the trials is to identify the most efficacious event resistant against rice stem borer and leaf folder. The number of events screened will be 566+ checks. There will be no use of herbicide and pesticide. Data to be collected include Dead hearts, white heads, Leaf folder damage levels and agronomic traits. The following reproductive isolation measures are proposed:

- 50 m isolation distance from any other rice crop;
- Border buffer zone with a rice variety all around the trial site; and
- 4 m high physical barrier with polythene sheet around the trial area.

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

1. **Plasmid description -** Vector used for transformation is a binary plasmid containing the pBR322 origin of replication with Kanamycin as bacterial selection marker. This antibiotic resistance gene does not integrate into the plant system as it is present outside of the integration region *viz*., T-DNA flanked by Right and Left Border. The gene of interest is driven by a proprietary Metahelix promoter and a 35S transcription terminator. Plant selection marker coding for *hph* gene resistant to Hygromycin is driven by CaMV promoter and a 35S transcription terminator. The T-DNA region encodes for gene of interest and the plant selection marker only and not the bacterial selection marker gene. The plasmid can be maintained in both *E.coli* and *Agrobacterium* with kanamycin selection.

2. **Transformation** - *Agrobacterium*-mediated transformation was used as the method of transformation of the gene of interest into the plant genome.

4.1.4 It was further noted that the IBSC has recommended the proposal on 1.6.2010. The proposal was recommended by the RCGM in its 91st meeting held on 27.7.2010, subject to

submission of nucleic acid sequence and site map 2010. The information submitted by the applicant was reviewed by the GEAC.

4.1.5 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request to conduct event selection trials on transgenic rice (*Oryza sativa*) events namely MHR01 to MHR566 containing *cry1Ac* and *cry1Ab* gene at one location within the institutional research farm.

4.2 Permission to conduct event selection trials on transgenic cotton (*Gossypium hirsutum*) events MIR-cotton (1 to 131) containing *cry1Ac* and *cry1EC* genes by M/s. Krishidhan Seeds Ltd., Jalna

4.2.1 The Committee considered the request of M/s. Krishidhan Seeds Ltd., Jalna to conduct event selection trials on transgenic cotton events MIR-cotton (1 to 131), containing *cry1Ac* and *cry1EC* genes. The event selection trials will be conducted within the Company owned land/research farm at Jalna. The seeds of T1 generation in Gossypium hirsutum were imported from NBRI with the approval of RCGM and advanced to T3 generation under contained condition in transgenic polyhouses.

4.2.2 The purpose of the trials is to identify the most efficacious event resistant against *Helicoverpa armigera and Spodoptera litura*. Data to be collected include plant growth, flowering, pest loads for target insects, molecular analysis, bioefficacy analysis etc. The number of events screened will be 131+ checks. The following reproductive isolation measures are proposed:

- 50 m isolation distance from any other cotton crop; and
- 5 border rows with a non-transgenic variety all around the trial site;

4.2.3 The Committee further reviewed information submitted by the applicant regarding gene construct and transformation method and noted that the transgenic cotton plants carry NBRI synthetic Cry1AC Gene and NBRI Cry1EC gene driven by bidirectional promoter PR-1(wound inducible) & Ca35 S to promoter. The T- DNA insert is in pCAMBIA 1301 vector backbone using Hygromycin resistance as selection marker.

4.2.4 It was further noted that IBSC has recommended the proposal on 8.6.2010. The proposal was also recommended by the RCGM in its 91st meeting held on 27.7.2010 wherein RCGM has recommended that artificial infestation of insects should be done as the area is not prone to pest infestation.

4.2.5 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request to conduct event selection trials on transgenic cotton events MIR-cotton (1 to 131), containing *cry1Ac* and *cry1EC* genes.at one location within the institutional research farm at Jalna.

4.3 Permission to conduct event selection trials on transgenic cotton (*Gossypium hirsutum*) variety namely ILK-Bt 77 (events ; ILK-Bt 77-1 to ILK-Bt 77-7) containing *cry1Ac* gene by Central Institute of Cotton Research (CICR), Nagpur.

4.3.1 The Committee considered the request of CICR Nagpur to conduct event selection trials on transgenic cotton variety namely ILK-Bt 77 (ILK-Bt 77-1 to ILK-Bt 77-7) containing *cry1Ac* gene at Nagpur.

4.3.2 The purpose of the study is to test the performance of new cry 1Ac events and protection against the bollworm across the season. The trials would be carried out at one location in an area of 15 sq. m to generate sufficient materials for BRL-I trial and biosafety experiments within the institutional research farm at Nagpur. The experimental data will be recorded on Cry 1Ac protein expression, seed cotton yield, other economical characters, maturity (duration), resistance to pest and disease and fiber technological properties. No herbicide will be used, however pesticide will be used for sucking pest as and when required. The following reproductive isolation measures are proposed:

- 50 m isolation distance from any other cotton crop; and
- Refugia crop (Pigeon Pea) all around the trial site;

4.3.3 The Committee also considered information on the gene construct and transformation method and noted that synthetic gene *cry 1Ac* (1.86kb) was cloned into pBinAR binary vector. BinAR, Bin 19 derivatives (binary vector: M. Bevan (84) NAR 12,8711) containing expression cassette for constitutive expression of chimeric transgene in plants. Expression cassette cloned in to Hind-III/Eco Rl sites of Bin 19. CICR has also clarified that the gene has been sourced from Director NRCPB. The gene is truncated Bt or cry 1 Ac gene, size 1860 bp with 25S CaMV Promoter. Genotypes used for the transformation is G hirsutum variety Angali (named as ILK-Bt 77). Transformation has been carried out by Agrobacterium mediation.

4.3.4 It was further noted that IBSC has recommended the proposal on 2.7.2010. The proposal was also recommended by the RCGM in its 91st meeting held on 27.7.2010.

4.3.5 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the request to conduct event selection trials on transgenic cotton (*Gossypium hirsutum*) variety namely ILK-Bt 77 (events ; ILK-Bt 77-1 to ILK-Bt 77-7) containing *cry1Ac* gene one location within the institutional research farm at Nagpur.

4.4 Permission to conduct Biosafety Research Level-1 (BRL-1 trials) on two transgenic corn hybrids namely (NK 6240 and NK 6607) containing GA21 event (*mepsps* gene) and one hybrid NK 6240 containing stacked genes Bt11XGA21 event *(cry1AbXmepsps* genes) by M/s. Syngenta Bioscience Pvt. Ltd., Pune

4.4.1 The Committee considered the request of M/s. Syngenta Bioscience Pvt. Ltd., Pune to conduct BRL-1 trials on two transgenic corn hybrids namely (NK 6240 and NK 6607) containing GA21 event (*mepsps* gene) and one hybrid NK 6240 containing stacked genes Bt11XGA21 event *(cry1AbXmepsps* genes) at six locations namely Banaras Hindu University Varanasi; Maharana Pratap Agricultural University, Udaipur; Directorate of Maize Research at Begusarai, Bihar; Tamil Nadu Agricultural University**,** Coimbatore**;** University of Agricultural Science, Bangalore and Syngenta Research Station at Aurangabad.

4.4.2 It was also noted that the applicant has submitted a single application for conducting BRL-trial on two hybrids (NK 6240, and NK 6607) containing GA21 event and one hybrid (NK 6240) with stacked event of Bt11xGA21 for a comparative evaluation on the performance of the event, with one gene and in stack combination. Trial locations, entries and treatment are same for all 3 hybrids.

4.4.3 The purpose of the study is to:

i. evaluate efficacy of the stacked event against specific lepidopteron insect pests of corn and application of Glyphosate herbicide;

ii. evaluate the efficacy of the GA21 event of corn against application of Glyphosate herbicide;
iii. conduct a comparative assessment of soil ecosystem weediness, morphology and phenotypic characters of transgenic corn and its non-transgenic counterpart hybrids.
iv. study impact of the event on NTOs and soil ecosystem;
v. produce sufficient plant material for further bio safety research of the event; and
vi. conduct protein expression studies of the transgenes in various plant parts at different time intervals.

4.4.4 It was noted that IBSC has recommended the proposal. The proposal was also recommended by the RCGM in its meeting held on 27.7.2010.

4.4.5 In view of the above stated facts and taking into consideration the recommendation of the RCGM, the Committee approved the conduct of BRL-I trials with transgenic corn hybrids (NK 6240 and NK 6607) containing GA21 event (*mepsps* gene) and one hybrid NK 6240 containing stacked genes Bt11XGA21 event *(cry1AbXmepsps* genes) at three locations maximum subject to the following conditions:

> "*Trials shall be conducted with an isolation distance of 300 m and physical barrier of 10 or 13 rows of African Tall Maize plants covering a distance of 6 to 7.8 metres all around the experimental plot area*".

4.5 Permission to conduct confined field trial for event selection on transgenic rice events (Hybrid Rice SPT maintainer events) generated using the SPT1 and SPT6 constructs by M/s. Dupont Knowledge Center, Hyderabad.

&

4.6 Permission to conduct event selection trials on transgenic rice events (Hybrid Rice SPT maintainer events) generated using the SPT1 construct by M/s. Dupont Knowledge Center, Hyderabad.

4.5.1 The Committee considered the following requests of M/s. Dupont Knowledge Center, Hyderabad to conduct event selection trials on 12 transgenic rice hybrids (*Oryza sativa* L) each containing the following events:

(a) The events generated using SPT1 construct namely; JH 15b, JH 16a, JH 16b, JH 17, JH 25b, JH 26a, JH 36 of BC3 containing ZM-AA1-Os-MSCA1-DsRED2 genes and SPT 6 construct namely;J6-1-45a, J6-1-8, J6-1-4d, J6-1-10b, J6-1-7d containing Os-MSCA1-ZM-AA1-DsRED2 genes will be evaluated.

(b) The events generated using SPT1 construct namely; DKC376, DKC 550, DKC653, DKC413b, DKC413a, DKC615, DKC1049b, DKC1049a, DKC839, JH35, and JH37 of BC2 generation and DKC 320 of BC1 generation containing ZM-AA1-Os-MSCA1-DsRED2 genes will be evaluated.

4.5.2 The objectives of the trials are to asses:

- frequency of transgene transmission through pollen in different events.
- seed produciblity of the event.
- expression levels of DsRed2 and ZM-AA1 in the tissues of hybrid rice SPT events.

4.5.3 It was noted that the event selection trials will be conducted within the Company's owned land/research farm Bangalore with the following reproductive isolation measures are proposed:

- 200 m isolation distance from the last row of transgenic plant on all four sides will be maintained.
- 8 ft tall polythene sheets as barriers between each event.

4.5.4 IBSC in its sixth meeting held on 9.7.2010 had approved the proposal. The proposal was also recommended by the RCGM in its 91st meeting held on 27.07.2010

4.5.5 During the deliberations, the Committee noted the following points:

(i) These events were developed by transforming M2O2 X T65 lines and then backcrossed into VIR54G9. All events are single copy events. This technology enables maintenance of male sterile female parental lines for use in hybrid seed production.

(ii) the present proposal pertains to hybrid rice seed production technology (SPT). It is a process that facilitates large scale production of non-genetically modified male sterile rice lines which can be used as female inbred parents for subsequent hybrid seed production.

(iii) This Hybrid Rice SPT is a transgene based process for the production of maintainer rice lines which which was generated by *Agrobacterium* mediated transformation of a male-sterile **(*Os-msca1*/ *Os-msca1*)** mutant rice line with the SPT1 construct that contains three genes namely ***Os-msca1 (mod1), Zm-AA1 and DsRed2(Alt1).*** These three genes are essential for the functioning of the Hybrid Rice SPT process.

(iv) Roles of Genes in the formation of male sterile female inbred rice lines:

- ***Os-Mscal(Mod1)***: Os-Msca1 is required for normal pollen development in rice. Homozygous mutation at this locus (***Os-msca1***/ ***Os-msca1)*** renders the plant incapable of producing pollen. Expression of *Os-Msca1(Mod1)* gene in the ***Os-msca1***/ ***Os-msca1 I*** genetice backgroung restores male-fertility to the plant.

- ***Zm-AA1:*** This gene when expressed in pollen leads to degradation of starch in pollen grains and thus renders the pollen grains incapable of fertilization. The ZM-AA1 protein in Hybrid Rice SPT maintainer rice lines is expressed in the developing pollen grains resulting in the depletion of starch and depriving the pollen of energy reserves required for successful pollen germination and pollen tube elongation. Hence any pollen containing the SPT insertion will be unable to complete the fertilization process and only the non-transgenic pollen remains viable and competent for fertilization.

- ***DsRed2 protein*** exhibits a high fluorescent intensity under appropriate illumination. Seeds expressing Ds Red2(Alt1) protein are fluorescent. These enable purification of transgenic Hybrid Rice SPT maintainer seed and quality control to identy and separate the Hybrid Rice SPT maintainer seed from the other seed.

(v) The expressed OS_MSCA protein restores fertility in transgenic Hybrid Rice SPT MAINTAINER. However, the ***Os-Mscal (Mod1)*** gene In Hybrid Rice SPT maintainer rice lines is hemizygous for the gene (***Os-Mscal (Mod1)/-)*** and as a result, oly half of the pollen produced contains the ***Os-Mscal (Mod1)*** gene

which also conjugated with the linked Zm-AA1 gene , which makes the half of the pollen infertile by degrading the starch by expressing the alpha-amylase enzyme. The remaining half of the pollen produced is non-transgenic, fertile and caries the endogenous recessive *Os-msca* mutant geneotype.

(vi) Therefore, when Hybrid Rice SPT maintainer is used as a pollinator to propagate the seed o non-transgenic male sterile female inbreds, the derived progeny retain their male-sterility genotype (***Os-msca1/Os-msca1***). These progeny also do not contain the inserted genes and therefore non-transgenic and used as a female parent for commercial hybrid seed production. The commercial hybrids produced using these male-sterile progenies are also non transgenic and fully fertile.

4.5.6 The Committee was of the view that the above technology being a relatively a new one, a detailed presentation from the applicant would be useful in understanding the genetic elements of the gene construct, molecular data on expression for alpha amylase in different tissues and data on segregation and sorting of trans-genic and non-transgenic rice seeds.

4.5.7 The Committee gave an opportunity to the applicant to make a presentation on their proposal and also provide the necessary clarification sought by the Committee. The following points were noted:

1. Both SPT1 and SPT6 constructs have essentially same genetic elements in T-DNA but the position of alpha amylase and MSCA gene is swapped in SPT6. That means there are two different constructs and events generated from these constructs will be different. This has been discussed elaborately in IBSC meeting and also mentioned in the application.

2. Molecular data on expression for alpha amylase in different tissues will be generated during the event selection trials

3. Segregation of transgenic and non-transgenic seeds will be done by selfing the SPT maintainer line. Data on segregation of transgenic and non-transgenic seeds in 1:1 ratio will be generated during EST trial.

4. Seed sorting system for segregating transgenic vs non-transgenic seeds is being developed in USA. SPT transgenic and non-transgenic seeds will be used to validate the seed sorter. The applicant further clarified that approval of GEAC will be obtained for exporting the seeds for this analysis.

4.5.8 After detailed deliberation, the Committee approved the proposal for only experimental event selection trials during which the applicant will generate detailed information on (i) molecular data on expression for alpha amylase in different tissues; (ii) data on segregation of transgenic; and (iii) non-transgenic seeds in 1:1 ratio and a validated seed sorting mechanism for segregating transgenic vs non-transgenic seeds will be developed.

4.5.9 The Committee further opined that decision on the next phase of trial (BRL-I) will be based on the data generated by the applicant during event selection trials and over-all policy on the issue of markers in transgenic plants.

4.7 Request for replacement of TC-2 maize hybrid with TC-3 maize in second year Biosafety Research Level (BRL-1) trials containing cry1F gene (Event TC1507 (DAS-01507-1) by M/s. Dow AgroSciences India Pvt. Ltd., Mumbai.

4.7.1 The Committee noted that the applicant has conducted BRL-I trials with transgenic maize (Zea mays) hybrids namely TC-1 and TC-2 containing *cry1F* gene (Event TC1507 (DAS-01507-1)) at Coimbatore and Bhavani Sagar in TNAU; and Balajigapade and Kathalgere in UAS, Bangalore during Kharif 2010 for biosafety, bio-efficacy and agronomy evaluation.

4.7.2 The present request of the Company is to allow them to plant TC-1 and TC-3 (instead of TC-2) hybrids during second season BRL-1 trials in Kharif 2010 as sufficient quantities of TC-2 seeds could not be produced during the seed production under confined green house conditions.

4.7.3 The applicant has also informed that both TC-1 and TC-3 hybrids were produced in Bangalore during Rabi 2009 under greenhouse condition with the approval of IBSC. It was further noted that RCGM has also recommended the request in its meeting held on 27.7.2010 on the ground that the GEAC is following an event based evaluation and approval process.

4.7.4 The Committee endorsing the views of the RCGM, approved the request to conduct second season BRL-I trials with TC-1 and TC-3 hybrids during Rabi 2010.

4.8 Permission to conduct event selection trials on transgenic tomato events ACS 3-9 and ACS 7-9 expressing antisense ACC synthase-2 gene for delayed ripening by National Research Centre on Plant Biotechnology (NRCPB), IARI, New Delhi

4.8.1 The Committee considered the request of NRCPB to conduct event selection trials on transgenic tomato events ACS 3-9 and ACS 7-9 expressing antisense ACC synthase 2 gene for delayed ripening in India at Genetics Farm, IARI land, New Delhi. The trials will be conducted at one location in an area of 1100 sq m.

4.8.2 The Committee further noted that genetically engineered transgenic tomato with events of ACS 3-9 and ACS 7-9 were developed with improved shelf-life and delayed ripening to minimize yield losses due to post harvest spoilage during storage or transportation and thereby improving the productivity of the crop. Transgenic tomato cv. Pusa Ruby having ACC synthase 2 gene in antisense orientation under the control of fruit-specific promoter LeACS4 (gene contract: Pa4a2ab), was developed via Agrobacterium-mediated transformation procedure, using nptII as the selection marker gene. The ACC synthase enzyme is responsible for catalyzing the synthesis of the phytohormone ethylene which triggers fruit ripening. The antisense version of the gene results knocking down the gene expression and failure of the protein synthesis. Accordingly, the ACC synthase enzyme is not synthesized and therefore not available for supporting the ethylene biosynthesis in fruits of transgenic plants. Hence, the expression of antisense ACC synthase gene will delay fruit ripening and extend shelf life of fruits. DNA sequence data and Plasmid Map (diagrammatic representation of antisense construct pA4A2AB) is given.

4.8.3 The objectives of the trials are to:

- Monitor different post-harvest ripening parameters of transgenic tomatoes from the breaker stage of fruit ripening corresponding to the wild type (non-transgenic) fruits.
- Evaluate shelf-life of transgenic tomatoes by firmness analysis of fruits.

- Study leaf senescence in transgenic tomatoes.
- Comparative assessment of morphology and phenotypic characters of transgenic tomato and non-transgenic wild type plants.
- Produce sufficient plant material to undertake bio-safety research and to generate data on feed and food safety.

4.8.4 The following reproductive isolation measures is proposed:

- 100 m isolation distance from the last row of transgenic plant on all four sides will be maintained.
- Non transgenic border rows will be maintained around the transgenic tomato crop.

4.8.5 IBSC has recommended the proposal. The proposal was also recommended by the RCGM in its 91st meeting held on 27.07.2010.

4.8.6 In view of the above stated facts and taking into consideration the recommendations of the RCGM, the Committee approved the event selection trials on transgenic tomato events ACS 3-9 and ACS 7-9 expressing antisense ACC synthase-2 gene within the institutional research farm at IARI, New Delhi.

Agenda item No.5: Other items:

5.1 Request from M/S Mahyco for seed production of non-Bt Roundup Ready Flex (RRF) Cotton hybrids (event MON 88913) incorporating cp4 epsps gene for use as Refugia during BRL-II trials in the North, Central and South Zones.

5.1.1 The Committee noted that the GEAC in its held on 12.5.2010 had approved the request of M/s Mahyco to conduct BRL-II Trials and seed production of Bollgard II X Roundup Flex (BGIIRRF) cotton hybrids containing cry1Ac & cry 2Ab and CP4EPSPS (events MON 15985X MON 88913) in the North, Central and South zones subject to the condition that the trials will be conducted under the supervision of Director CICR, Nagpur and as per the protocols prescribed by him.

5.1.2 Director CICR has directed M/s Mahyco to plant non-Bt Roundup Ready flex (RRF) cotton hybrids (Event MON 88913) incorporating the cp4epsps gene as refugia in RRF BRL-II trials. Accordingly, the applicant has requested approval of GEAC for seed production of non-Bt Roundup Ready flex (RRF) cotton hybrids (Event MON 88913) incorporating the cp4epsps gene for the use as a refugia in an area of 16 acre in each zone (total 48 acre) for North, Central and South zone respectively.

5.1.3 The Committee noted that the Roundup Ready flex (RRF) in cotton hybrids (Event MON 88913) has not been approved for environmental release and, therefore, rejected the request of the applicant for seed production of non-Bt Roundup Ready flex (RRF) cotton hybrids (Event MON 88913) incorporating the cp4epsps gene as refugia in RRF BRL-II trials.

5.2 Permission for Export of Bt cotton hybrid seed to Pakistan by M/s Bioseed Research India Ltd. Hyderabad.

5.2.1 The Committee considered the request from M/s Bioseed Research India Ltd. Hyderabad to export Bt cotton BGII cotton hybrids containing *cry 1Ac and cry 2Ab genes* (Mon-15985) 15 numbers to Monsanto Pakistan Agritech Pvt. Ltd, Lahore namely:

- 6488-2, 2510-2, 2113-2, 563-2 and 6317-2 The proposed shipment will be total 200 Kg. (200 kg x 5) of BG II and

- 811-2, 812-2, 813-2,814-2, 815-2, 816-2, 817-2,818-2,819-2, 820-2 The proposed shipment will be total 20 Kg. (20 kg x10) of BG II

5.2.2 The intended purpose of the export is for conducting multi-locational field trials in different agro climatic zone in Pakistan. Bollgard II (Mon 15985) has been commercialized in India since 2006. The applicant has submitted a copy of the license agreement with M/s Monsanto Pakistan to import Bollgard II cotton hybrid seed for field trials.

5.2.3 After detailed deliberation, the GEAC conveyed 'No Objection' for export of Bt cotton BGII cotton hybrids containing *cry 1Ac and cry 2Ab genes* (Mon-15985) 15 numbers to Monsanto Pakistan Agritech. Pvt. Ltd, Lahore subject to the following conditions:

- Approval of the Pakistan National Biosafety Committee in accordance with the Pakistan Biosafety Rules, 2005 and National Biosafety Guidelines, 2005;

- Approval from the National Biodiversity Authority, Chennai

5.2.4 The need for approval of GEAC for export of approved events was briefly discussed wherein the Committee opined that the matter may be brought to the GEAC as an agenda item in the next GEAC meeting.

Agenda Item No 6: Any other matter with the permission of the Chair.

6.1 Permission to conduct elite event selection trials on Glytol cotton (*Gossypium hirsutum*) hybrids during Kharif 2010 by M/s. Bayer Bioscience Pvt. Ltd. Gurgaon

6.1.1 The Committee noted that decision on the proposal was deferred on want of the following information from the applicant:

i. What is the region of homology between the intermediate cloning vector and the non-oncogenic Ti plasmid?

ii. What is the structure of the co-integrate plasmid?

iii. What is the structure of the T-DNA region in the co-integrate plasmid?

iv. What is the evidence that only the T-DNA region from the co-integrate plasmid is introduced into the cotton plants in which event selection trails will be conducted?

6.1.2 The Committee considered comments received from Dr. Ramesh Sonti and Dr. P. Ananda Kumar and noted that clarification provided by the applicant is in order. To a query on why in the original application, the applicant had indicated that pTEM2 as an intermediate cloning vector, the applicant has clarified that it is an inadvertent error. The Committee opined that in future the Company should exercise due diligence in providing the correct information to the regulatory authority.

6.1.3 After detailed deliberations and taking into consideration the views of the expert members the Committee approved event selection trials on Glytol cotton (*Gossypium hirsutum*) hybrids during Kharif 2010 at one location

6.2 Request to conduct Bio-safety Research level-1 (BRL-1) trials under confined conditions **on two transgenic maize hybrids namely 30V92HR and 30B11HR with indigenously produced seeds containing *cry1F & PAT* and *CP4EPSPS* genes (TC1507 x NK603 (DAS-01507-1 x MON-00603-6) from Rabi 2009-2010 to Rabi season 2010-11 by M/s. Pioneer Overseas Corporation, Hyderabad.**

6.2.1 The Committee noted that the GEAC in its 98th meeting held on 9.12.2009 had approved to conduct BRL-1 trials under confined conditions on two transgenic maize hybrids namely 30V92HR and 30B11HR with indigenously produced seeds containing *cry1F & PAT* and *CP4EPSPS* genes (TC1507 x NK603 (DAS-01507-1 x MON-00603-6) at 4 SAUs during Rabi 2009-10.

6.2.2 It was noted that the present request is for conducting BRL-I trials during Rabi 2010-11 with the same transgenic hybrids with same objective and at same locations. It was noted that the BRI-I trials during Rabi 2009-2010 could not be conducted due to the non-availability of proper experimental sites at approved State Agricultural Universities

6.2.3 It was further noted that the RCGM in its 92nd meeting held on 25.08.2010 had approved the request of the applicant and extended the validity of the permission granted on 29.12.2009 for the conduct of BRL-I trials during Rabi 2010-11.

6.2.4 In view of the above stated facts, GEAC approved the conduct of BRL-I trials with transgenic corn hybrids expressing stacked event of TC1507 and NK603 for 2010-2011 Rabi season.

6.3 Permission to conduct Biosafety Research Level-1 (BRL-1) trials, seed production and Environmental safety studies (Crossability studies) on transgenic mustard (*Brassica juncea)* hybrid DMH-11 containing *barnase, barsar and bar* genes [events bn 3.6 (Barnase line) and modbs 2.99 (Barstar line)] during third week of October, 2010 by University of Delhi South Campus, New Delhi.

6.3.1 The Committee considered the request of University of Delhi, South Campus **(UDSC),** New Delhi to conduct Biosafety Research Level-1 (BRL-1) trials on transgenic mustard (Brassica juncea) hybrid DMH-11 containing barnase, barsar and bar genes [events bn 3.6 (Barnase line) and modbs 2.99 (Barstar line)] and its parental lines Varuna event bn 3.6 and modbs 2.99 under the overall coordination of Directorate of Research on Mustard and Rapeseed, Bharatpur at six locations namely Experimental Farm, Punjab Agricultural University, (PAU, Ludhiana); Agricultural Research Station experimental Farm Navgaon (RAU, Bikaner); Agricultural Research Station Sriganganagar (RAU, Bikaner); KVK, Kumher (Bharatpur); Oilseeds Research Farm, Kalyanpur (Kanpur) and ZARS, Morena during third week of October, 2010.

6.3.2 The Committee also considered information on gene construct and transformation method. It was noted that the event bn 3.6 (Barnase line) and event modbs 2.99 (Barstar line) were selected in RLM 198 and Varuna variety of B. juncea respectively by UDSC. Event bn 3.6 and modbs 2.99 were backcrossed into variety Varuna and east-European type mustard line EH-2 respectively which were further crossed to form F1 hybrid DMH-11. The following reproductive isolation measures are proposed:

- Transgenic parents –Varuna barnase (event bn3.6) and EH2 bastar (event modbs 2.99), One non transgenic parent (EH2), one national check (varuna) and one Check would be planted along with the transgenic mustard hybrid DMH-11. and;

- 50 m isolation distance from planting area on all sides.

6.3.3 The purpose of the trials is to generate biosafety data with focus on environmental safety assessment parameters viz. reproductive and survival biology, weediness potential and non target adverse effects

6.3.4 The Institution has also requested to conduct Experimental Seed Production at a limited scale in 100 sq. m area for further use in confined field trials at their own research farm located at Jaunti Village and to conduct Crossability studies as part of environmental safety assessment) with transgenic mustard (Brassica juncea) hybrid DMH-11 containing barnase, barsar and bar genes [events bn 3.6 (Barnase line) and modbs 2.99 (Barstar line)] Delhi during third week of October, 2010 at their own research farm located at Bawana, Delhi to purpose to study the cressability of the transgenic Brassica juncea hybrid DMH-11 with related Brassica species i.e.B.rapa (toria, yellow sarson, brown sarson), B.nigra, B.oleracea (early types), B.napus, B.carinata, B.touneforti. Eruca sativa and Raphanus sativus.

6.3.5 It was further noted that IBSC has recommended the proposal on 24.8.2010. and RCGM has also recommended the request of the UDSC for conduct of BRL-I trial and limited seed production and crossibility studies. in its 92nd meeting held on 25.08.2010. to conduct Biosafety Research Level-1 (BRL-1) trials

6.3.6 After detailed deliberation and taking into consideration the recommendation of RCGM., the Committee approved the request of UDSC to conduct BRL-I trials on transgenic mustard (*Brassica juncea)* hybrid DMH-11 containing *barnase, barsar and bar* genes [events bn 3.6 (Barnase line) and modbs 2.99 (Barstar line)] at two to three locations. The Committee also approved the conduct of crossibility studies and limited seed production within the institutional research farm located at Jaunti Village and Bawana, Delhi respectively.

6.4 Permission to conduct Biosafety Research Level-1 (BRL-1) trials on transgenic mustard (*Brassica juncea L)* varieties namely Pusa Jaikisan and Varuna containing osmotin gene (event Omb5-B) confers tolerance to drought stresses in *Brassica juncea* during first week of November, 2010 by National Research Centre on Plant Biotechnology, IARI Campus, New Delhi

6.4.1 The Committee considered the request of National Research Centre on Plant Biotechnology, IARI Campus, New Delhi to conduct Biosafety Research Level-1 (BRL-1) trials on transgenic mustard (Brassica juncea L) varieties namely Pusa Jaikisan and Varuna containing osmotin gene (event Omb5-B) that confers tolerance to drought stresses in Brassica juncea at Genetics Farm, IARI Campus, IARI, New Delhi during first week of November, 2010

6.4.2 The objectives of the proposed BRL-1 trials include the following:

a) To evaluate the field efficacy of the transgenic event in terms of the level of tolerance to abiotic stresses corresponding to the wild type (non-transgenic) plants.

b) Comparative assessment of morphology and phenotypic characters of transgenic mustard and non-transgenic wild type plants.

c) To undertake biosafety research and to generate data on feed and food safety.

6.4.3 The Committee noted that RCGM had approved the request of the Company in its 92nd meeting held on 25.08.10 subject to the submission of the IBSC minutes. Subsequently, IBSC has approved the proposal in its meeting held on 4.09.2010. The issue on whether RCGM can approve a proposal without the submission of IBSC minutes was raised by one of the members. The Committee opined that only proposal approved by the IBSC should be considered by RCGM. Any application without the IBSC approval may be treated as incomplete and rejected for non-submission of requisite information.

6.4.4 After detailed deliberation and taking into consideration the recommendation of RCGM, the Committee approved the request of National Research Centre on Plant Biotechnology to conduct BRL-I trials on transgenic mustard (*Brassica juncea L)* varieties namely Pusa Jaikisan and Varuna containing osmotin gene (event Omb5-B) confers tolerance to drought stresses in *Brassica juncea* at two to three locations within the institutional research farm of IARI.

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