REVISED GUIDELINES FOR RESEARCH IN TRANSGENIC PLANTS & GUIDELINES FOR TOXICITY AND ALLERGENICITY EVALUATION OF TRANSGENIC SEEDS, PLANTS AND PLANT PARTS, 1998

Department of Biotechnology, Ministry of Science and Technology, Govt. of India

1. INTRODUCTION

The revised present document is meant for the researchers in the country who are involved in recombinant DNA research on plants. Earlier the Department of Biotechnology in January 1990 issued a compendium of guidelines under the title "Recombinant DNA Safety Guidelines". A revision was made in 1994 under the title "Revised Guidelines for Safety in Biotechnology". The current guidelines have been developed in the light of enormous progress that has been made in recombinant DNA research and its widespread use in developing improved microbial strains, cell lines and transgenic plants for commercial exploitation.

2. COVERAGE OF THE REVISED GUIDELINES

The current guidelines cover areas of recombinant DNA research on plants including the development of transgenic plants and their growth in soil for molecular and field evaluation. The guidelines also deal with import and shipment of genetically modified plants for research use only.

3. STATUTORY BODIES DEALING WITH THE RECOMBINANT DNA WORK

In accordance with the Notification No. GSIR 1037 (E) dated 5th December, 1989 of the Ministry of Environment & Forests which empowers the Review Committee on Genetic Manipulation (RCGM) to bring out manuals of guidelines specifying procedure for regulatory process with respect to activities involving genetically engineered organisms in research use and applications including industry with a view to ensuring environmental safety, the present changes in the procedures are being made. These changes are made reiterating the powers conferred on the RCGM to lay down procedures restricting or prohibiting production, sale, importation and use of genetically engineered organisms or cells as are mentioned in the attached schedule of the above mentioned notifications.

A. IBSC (Institutional Biosafety Committee)

i. The IBSC is the nodal point for interaction within an Institute/ University/commercial organisation involved in r-DNA research for the implementation of the recombinant DNA guidelines. As such, in the first instance, it is necessary that the organisations intending to carry out research activities involving genetic manipulation of microorganisms, plants or animals should constitute their IBSC in accordance with the procedures in vogue and as informed to the public through the above notification. All recombinant research carried out by the organisation should have a designated Principal Investigator (P.I.). It would be the duty of the P.I. to apprise its IBSC about the nature of the experiments being carried out. Depending upon the category of the experiments as narrated on in the present guidelines the P.I. Can inform the IBSC about the recombinant experiments, seek permission of IBSC before starting the experiments or seek the permission of the RCGM through its IBSC in cases where the risks involved in the experiments are considered to be of higher magnitude having the potential of polluting/endangering the environment, the biosphere, the eco system, the animals and the human beings.

The Department of Biotechnology in January 1990 enumerates the duties of the IBSC in pages 15-16 of the original "Recombinant DNA Safety Guidelines" prepared.

B. RCGM (Review Committee on Genetic Manipulation)

i. The RCGM is functioning in the Department of Biotechnology to monitor the safety-related aspects of ongoing research projects involving genetically engineered organisms.

ii. The RCGM shall include representatives of a) Department of Biotechnology; b) Indian Council of Medical Research; c) Indian Council of Agricultural Research; d) Council of Scientific and Industrial Research; and e) others experts in their individual capacity. RCGM may appoint subgroups to monitor specific projects.

iii. The RCGM would review all the reports of all approved on-going research projects involving high-risk category and controlled field experiments.

iv. The RCGM or its constituted subgroups shall visit the site of experimental facilities periodically, where projects with biohazard potential are being pursued and also at a time prior to the commencement of the activity to ensure that adequate safety measures have been taken as per the guidelines.

v. The RCGM would issue the clearance for import/export of etiologic agents and vectors, transgenic germplasms including transformed calli, seeds and plant parts for research use only.

vi. The RCGM shall meet at least twice in a year.

vii. For research in recombinant DNA work-involving risks categorised as category-III and above in this revised document the permission of the RCGM through the Department of Biotechnology must be obtained by the P.I. Before conducting the research work.

viii. RCGM can authorise applicants (P.I.s) to conduct limited field trails in multi locations in the country. The design of the trial experiments is either provided by the RCGM or it may approve the protocol designed by the P.I. The protocol will seek answers related to animal and human health. Data should also be generated on economic advantage of the transgenics over the existing varieties.

ix. RCGM can, if required, direct the applicants to generate toxicity, allergenicity and any other relevant data on transgenic materials in appropriate systems. RCGM may design or approve a protocol for conducting experiments to seek answers to the above.

x. The RCGM can put such conditions as would be required to generate long term environmental safety data from the applicants seeking release of transgenic plants into the open environment, and who have complied with initial safety evaluation.

xi. RCGM can approve applications for generating research information on transgenic plants. Such information may be generated in contained green house as well as in very small plots, as research needs to be conducted in such environment for seeking answers to specific environmental safety issues emanating from the use of transgenic plants. The small experimental trials should be limited to a total area of 20 acres in multi-locations in one crop season. In one location where the experiment is conducted with transgenic plants, the land used should not be more than one acre. Any experiment beyond the above limits in one crop seasons would require the approval of the Genetical Engineering Approval Committee (GEAC).

4. CATEGORIES OF GENETIC ENGINEERING EXPERIMENTS ON PLANTS AND THEIR NOTIFICATIONS

A. CATEGORY I, routine recombinant DNA experiments

This category includes routine cloning of defined genes, defined non-coding stretches of DNA and open reading frames in defined genes in E. coli or other bacterial and fungal hosts which are GENERALLY CONSIDERED AS SAFE (GRAS) to human, animals and plants. A list of such microorganisms will be prepared by the RCGM and shall be made available to the P.I. on request.

This category involves experiments in the lab in contained environment and includes the following:

i. Routine cloning of defined DNA fragments of microbial, animal and plant origin in GRAS organisms.
ii. Transfer of defined cloned genes into Agrobacterium;
iii. Use of defined reporter genes to study transient expression in plant cells to study genetic transformation conditions;
iv. Molecular analysis of transgenic plants grown in-vitro.

Categories I experiment need only intimation to the IBSC in the prescribed proforma (available with the RCGM secretariat).

B. CATEGORY II

This category includes lab and green house/net house experiments in contained environment where defined DNA fragments non-pathogenic to human and animals are used for genetic transformation of plants, both

model species and crop species and the plants are grown in the green house/net house for molecular and phenotypic evaluation.

This category includes the experiments described below :

1. Transgenics with constitutive, tissue specific and chimeric promoters used for experimenting expression of defined DNA fragments.
2. Marker genes extensively used in genetic transformation of plants in lab and green house/net house experiments.
3. Lab and green house/net house experiments with plants with herbicide resistance conferring genes;
4. Lab and green house/net house experiments with plants using heterologous genes which confer resistance to biotic and abiotic stresses (i.e. genes like chalcone synthase, heat shock proteins, chitinase, protease inhibitors etc);
5. Lab and green house/net house experiments with genes from plants, animals and microbial sources that would confer resistance to plant pathogens.
6. Lab and green house/net house experiments with transgenics with genes for the production of antibodies.
7. Green house/net house experiments with transgenics with transposable elements for gene tagging in crop species.

Permission for performing Category II experiments will be provided by IBSC. The decision of the IBSC would be intimated to the RCGM before execution of the experiments and RCGM would put this information on record.

C. CATEGORY III & ABOVE

This category pertains to high risk experiments where the escape of transgenic traits into the open environment could cause significant alterations in the biosphere, the ecosystem, the plants and animals by dispersing new genetic traits, the effects of which can not be judged precisely. All experiments conducted in green house and open field conditions not belonging to the above Category II types, would fall under Category III risks. Such experiments could be conducted only after clearance from RCGM and notified by the Department of Biotechnology:

5. CONTAINMENTS

Different levels of containment are prescribed for the three different categories of rDNA experiments.

1. Category I experiment should be performed using routine good laboratory practices (See Appendix I for details)
2. For Category II experiments dealing with evaluation of transgenics in green house/net house, the designs for the contained facility shall be as described in Appendix II. The transgenic experiments of Category II risks will have to be carried out in green house/net house, the specification of which is significantly stringent to ensure arrest of transgenes within the contained facility.
3. For Category III experiments in green house/net house, the later needs to be designed as indicated broadly in Appendix II. The specifications of the green house/net house have been designed to ensure near complete isolation of the facilities from the open environment; care has also been taken to prevent the entry of insects into the green house/net house facility.

For limited field experiments in the open environment, the RCGM would provide for and/or would approve the design of the experimental field plots.

6. MONITORING AND EVALUATION MECHANISMS FOR GREEN HOUSE/ NET HOUSE EXPERIMENTS AND LIMITED FIELD TRIALS IN THE OPEN ENVIRONMENT

The RCGM can bring out manuals of Guidelines specifying procedures for regulatory process with respect to activities involving genetically engineered organisms in research and applications to ensure environmental safety. To monitor, over a period of time, the impact of transgenic plants on the environment, a special Monitoring cum Evaluation Committee of the following constitution will be set up by the RCGM. The Committee shall have the following constitution.

a)	Chairman of the Committee	:	Secretary, DBT & Secretary, DARE shall jointly discuss and elect a leader of the committee.
b)	Eminent Plant Biotechnologists	:	To be nominated by RCGM, 3-4 Nos.
c)	Seed Technologies	:	To be nominated by ICAR, 2-3 Nos.
d)	Plant Breeders	:	To be nominated by ICAR, upto 2 Nos.
e)	Plant Ecologists/Environmentalists	:	To be nominated by RCGM, upto 2 Nos.
f)	Nominee of NBPGR	:	To be nominated by ICAR.
g)	Nominee of MOE&F	:	To be nominated by the Chairman, GEAC
h)	Member-Secretary	:	Member-Secretary, RCGM

This committee will undertake field visits at the experimental site/s. The committee shall be guided by the RCGM on the design of field experiments and on the preparation of formats for collecting scientific information on plants in green house/net conditions as well as in limited field trials. Based on the on-the-spot situation the committee can suggest remedial measures to adjust the original trial design and assist the RCGM in collecting, consolidating and analysing the field data for evaluating the environmental risks emanating from the transgenic plants. This committee shall also collect or cause to collect the information on the comparative agronomic advantages of the transgenic plants. From time to time, the committee shall advise the RCGM on the risks and benefits from the use of the transgenic plants put into evaluation. Trials will be done for at least one year with minimum four replications and ten locations in the agroecological zone for which the material is intended. The biological advantage of transgenic will have to be clearly enumerated by the applicant, the Institution, the University or the Industry. The latter would recommend those transgenics, which would be found to be environmentally safe and economically viably by the RCGM, to the Genetic Engineering Approval Committee for consideration for release into the environment.

7. BIOSAFETY ASPECTS OF THE TRANSGENIC PLANTS

Experiments are designed to systematically identify the hazards, to access to risks and to take step to manage the risks by applying logically valid strategies, to systematically identify the hazards and to assess the risks; the information on the following aspects would be required to be generated.

I. Characteristics of the donor organisms providing the target nucleic acids. These may include the following:

1. Name of the donor organism with its identification characteristics with relevant reference to published information if any.
2. Pathogenically and toxicity characteristics to plants and animals.
3. Allergenicity characteristics to human alongwith of the allergenic substances, wherever possible.
4. The geographical origin of the organisms, its distribution pattern and survival mechanisms.
5. The method of transfer of its genetic materials to other organisms.

II. Characteristics of the vectors used : These may include the following :

1. The origin, identity and habitat of the vectors used.
2. The sequence, frequency of mobilisation, specificity and marker genes if any, present in the vectors.
3. The abilities of the vectors to get established in other hosts; the hosts are also to be specified.

III. Characteristics of the transgenic inserts : These may include the following :

1. The specific functions coded by the inserted nucleic stretches including the marker gene inserts.
2. The expression of the nucleic acid products and their activities/properties.
3. The toxicity of the expression products on the host plant, if any.
4. The toxicity and allergenicity of the nucleic acid products to human and animals.

IV. Characteristics of the transgenic plants : These may include the following :

1. Methods of detection of the transgenic plant in the environment.
2. Methods of detection and characterization of the escaped transgenic traits in the environment.
3. Toxicity and pathogenicity of the transgenic plants and their fruits to other plants in the ecosystem and the environment.
4. Possibility of and the extent of transgenic pollen escape and pollen transfer to wild near relatives, and the consequences to the environment.
5. Pathogenicity, toxicity and allergenicity of the transgenic plants and their fruits to human and animals.

Information on many of the above questions may already be available. Many questions may however be required to be investigated and answers found out, for which appropriate new experiments would have to be designed to gather data. For generating toxicity and allergenicity data, standard protocols devised by international agencies could be used. The Indian national toxicological laboratory like the Industrial Toxicology Research Centre, Lucknow could be consulted to generate appropriate protocol for these purposes.

For minimizing the risk arising from the limited release of transgenic plants, the following may be taken into consideration :

1. Special separation for isolation, for preventing reproduction/fertilisation and seed setting.
2. Biological prevention of flowering by making use of sterility properties etc.
3. Human intervention for the removal of reproductive structures of flowers.
4. Controlling the reproductive structures of transgenic plants like the seeds and the plant propagules from unaccounted spread.
5. Controlling and destroying volunteer plants from the experimental field.
6. To take into account the proximity to human activity in case the transgenic plants have allergenic properties to human and animals.
7. Appropriate training of field personnel responsible for handling the transgenic plants.
8. Plans for handling unexpected events.
9. Documentation of previous published information, if any, including any documented evidence of effects of release to ecosystem.

Thorough comparison with national checks for productivity and susceptibility/resistance to biotic and abiotic stresses will have to be made.

All the information as above are to be documented in the form of a document which would be called the registration document.

8. IMPORT AND SHIPMENT OF TRANSGENIC GERMPLASM FOR RESEARCH PURPOSES:

Clearance for import of transgenic material, for research purposes would be provided by the RCGM. The RCGM will issue an import certificate after looking into the documents related to the safety of the material and the national need. The RCGM will take into consideration the facilities available with the importer for in-soil tests on the transgenic material. The importer of a transgenic material may import the material accompanied by an appropriate phyto-sanitary certificate issued by the authority of the country of export, and such import may be routed through the Director, NBPGR on the basis of the import permit issued by the DBT, based on the recommendations of the RCGM. The import certificate would be cancelled if NBPGR would not provide the phyto-sanitory certificate. NBPGR will provide information on the time that is required for phyto-sanitary evaluation. These evaluations will be done in a time-bound manner in presence of the agents of the institutes or the commercial organisations that are importing the material, if they so desire. Parts of the seed material will be kept at NBPGR in double lock system in the presence of the importer. This lot of seed will act as a source material in case of any legal dispute.

APPENDIX – I

GOOD LABORATORY PRACTICES

1. Use a pipettor for all the solution transfers. No mouth pipetting.
2. Plug pipettes with cotton.
3. Do not blow infectious material out of pippetes.
4. Do not prepare mixtures of infectious material by bubbling expiratory air through the liquid with a pipette.
5. Before and after infecting an animal, swab the site of injection with a disinfectant.
6. Sterilise discarded pipettes and syringes in pan where they were first placed after use.
7. Before centrifuging, inspects tubes for cracks. Inspect the inside of the Turin cup for rough walls caused by erosion or adhering matter. Carefully remove all bits of glass from the rubber cushion.
8. Use of centrifuge tunnion cups with screw caps or equivalent.
9. Avoid decanting centrifuge tubes; if you must do so, wipe off the outer rim with a disinfectant. Avoid filling the tube to the point that the rim ever becomes wet with culture.
10. Sterilise all contaminated material before discarding.
11. Periodically, clean out deep-freeze and dry-ice chests in which cultures are stored to remove broken ampules or tubes. Use rubber glovers and respiratory protection during the cleaning.
12. Avoid smoking, eating and drinking in the laboratory.
13. Do not reuse plasticware that has been used for PCR, recombinant DNA work and plant transformation work.
14. Sterilise all the plasticware before discarding it.
15. Burn all the transgenic material in an incinerator after observations have been taken.

APPENDIX – II

MODEL PLAN FOR THE CONSTRUCTION OF
A GREEN HOUSE/NET HOUSE FOR EXPERIMENTS USING TRANSGENIC PLANTS

Frame Structure : The structure should be made from galvanised mild steel designed to with stand wind loading of not less than 100 km/hour. The method of affixing the polythene film cover to the frame should be strong enough to with stand similar wind velocities. The base may be constructed with bricks and cement or with any durable structure up to a height of 1.5 to 2 feet from the ground level so as to isolate the land inside the framed structure from the outside land.

Optimum size of unit : The recommended minimum size of the unit would be 1000 to 1500 cubic meters. In dimensions each such unit may be 30 meters long 13 meters wide and having and under the gutter height of about 3 to 4.5 meters from the base. The plan view as well as the side view of a multi span unit with double door entry recommended for an optimum size unit is enclosed along with this appendix-II. It is recommended that all the green house structures should have double door entry as indicated in the enclosed drawings, and the span of the area for the double door entry can be kept as 5 to 6 meters in length and about 3 meters in width along with height maintained commensurate with the main structure of the unit. The main entrance may be optionally be provided with an air curtain. The outer door shall be only one panel of flush door opening inside the buffer area and the inside doors may be more than one (two sliding doors have been shown in the drawing). In case sliding doors are not installed, the inside doors should be of one panel each, opening inside the buffer area only. The entry wall can be utilised for housing the suction fans as shown in the drawing while the opposite wall can be mounted with evaporation pads (shown in the drawing). The optimum sized unit recommended above would provide a growing area of about 350 sq. meters, allowing 10% for path ways. This unit have a Volume of about 1100 - 1200 cubic meters. Such a unit would be able to maintain a stable temperature, the desired humidity with adequate and ample air circulation.

Plastic film covering : It is recommended that the area covering the frame should be of 200 micron (800 gauge) thickness, UV stabilised polymer film. Such materials are expected to have a life span of 4 to years. All coverings should be double film covering on all surfaces to give double UV filtration and a more stable temperature control. The roof covers are likely to be inflated by the action of blower fans, thus maintaining a cavity throughout the unit. In addition to its suggested that an internal separation wall can be constructed to bifurcate the spans if there are more than one, which can be done by fixing the plastic films to the securing rails. With in the whole unit facilities can thus be provided for separate crop studies.

Fan, Pad system and Filter screens : An evaporative cooling system will be required to enable the maintenance of stable temperature gradient from the site of evaporating pad to the suction end. The surface are of the cooling unit will depend upon

the overall all size of the structure. If the unit exceeds 30 meters in length then the temperature variation through out the length of unit may be such than an even temperature may not be maintainable even with the introduction of turbo circulation fans. The dimensions of the evaporation pad required to obtain a temperature 15 degree centigrade below ambient for a give volume of green house can be calculated from the following approximate equation.

Pad area (P) = Length X Width X Height, the whole divided by 94.85
Where P is in sq. Meter area.

As an example it is stated that a unit having the dimensions of 30 meters X 13 meters X 3 meters requires a pad area of not less than 12.35 Sq. meters. As most pad units are constructed to order, it is expected that it would not be difficult to have the pad areas of correct size.
All external surfaces of the pad should have filter screens of at least a 40 X 30 mesh net covering made from durable plastic material.

The fans required for a unit of above dimensions, to be housed at the other end of the unit should be about 61 centimeters (24 inch) in diameter with low noise and high C.u. ft./min (CFM) air circulation capacity, with four numbers to be installed per unit. It is recommended that motors with 1.5 H.P. with three phase may be installed which is slightly over designed but which is expected to have more life span and there fore substantial saving on replacements. Compromises can be made by installing 1 H.P. three phase motors, but this may need more maintenance. The fan units should have 40 X 40 mesh durable plastic screen fitted to the out side of the external surface. Each motor unit can be connected to one semi automatic temperature controlled which should shut down the fan as and when the temperature drops below the required levels. Such designs are available in the market.

Blower fans are required to be fitted on the each roof section which will inflate the top roof sheet. These fans must also to be fitted with 40 X 40 mesh durable plastic screen on the induction side to prevent any pollen evacuat ion. As these fans are expected to be constantly in operation it is recommended that these should be fitted with bearings and not with bust type.

It is essential to have circulation fans within the green house to ensure that a uniform temperature is maintained though out the growing area. The number and the positioning will however depend upon the external conditions and therefore may vary from place to place. The manufacturer may be consulted for selecting the correct number.

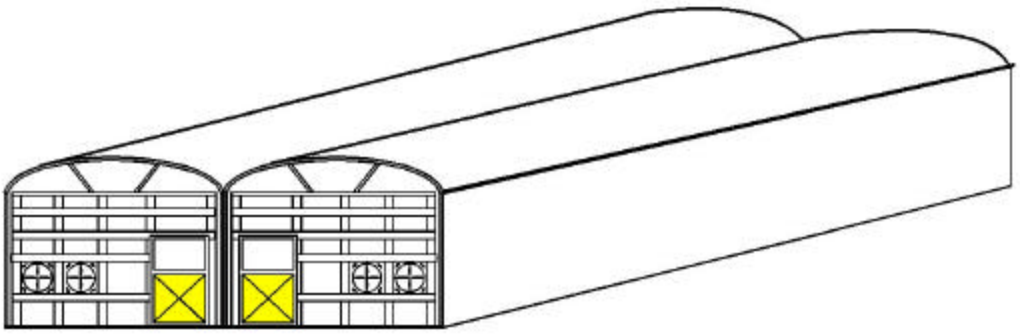
Irrigation : Full over head irrigation systems are available and can be installed. In smaller houses it would be advisable to carryout the watering manually as regulation of humidity is difficult to maintain through over head irrigation system because any extra watering will increase the humidity level. In line feeding units can be installed to take care of the nutrient requirements of the plants. A water tank needed to supply water to the pads and irrigation may be installed slightly below the ground level to avoid direct influence by sun or solar heat. The water will therefore remain cool.

Proposed positioning : The location and the orientation of the unit is of significant importance. The fans should not be positioned in a manner that they below directly to wards the plants. Electricity and water are continuously required. Therefore these must be positioned within a reasonable reach of the unit to keep costs down. The area selected for the unit must be flat, and as far as possible leveled to accommodate the unit plus approximately 2 metros off around the outside. It would be useful to provide a drainage system around the unit at suitable lower levels to enable the drainage of extra water. A suitable drain off area is also recommended to enable the extra water running off from the gutters; the drain off area may be more than 10 meters away from the unit.

Views showing the Different aspects of Playhouse/Greenhouse : Five diagrams showing schematically one recommended unit of the dimension 30 meters X 13 meters X 3 meters (Length X Bre adth X Gutter height, excluding the dome height) are appended at enclosures I to V. The installers can install units bigger than the one suggested above. However, they have to ensure that all the safety precautions namely, installation of double doors, use of durable structures for the framework, use of at least 200 micron (800 gauge) plastic films in double coverings are used in the construction. Further, all the outlets would have to be secured by applying 40X40 mesh durable plastic coverings as indicated above.

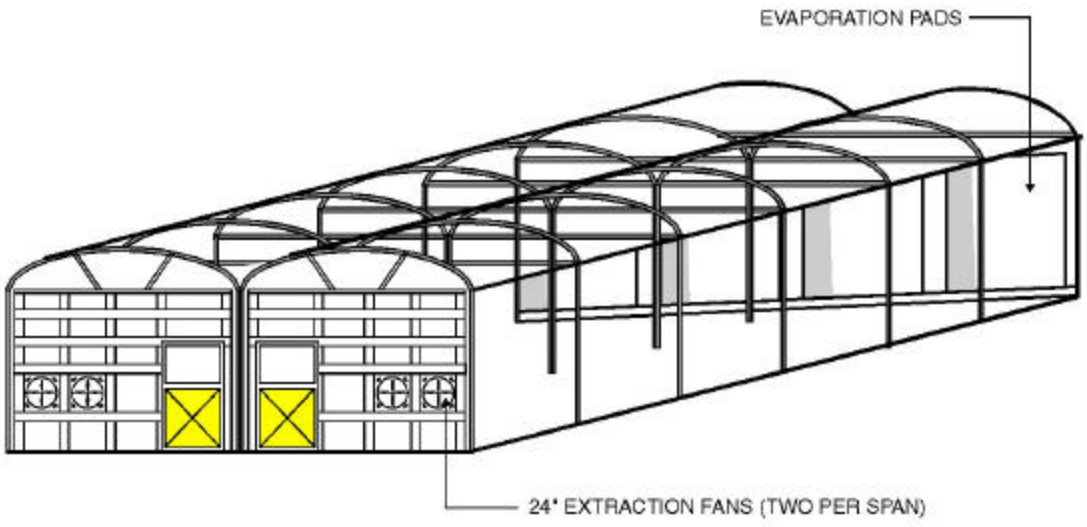
DOUBLE SPAN ENCLOSED UNIT FOR TRANSGENIC PLANT PRODUCTION

WITH FAN & PAD COOLING SYSTEM

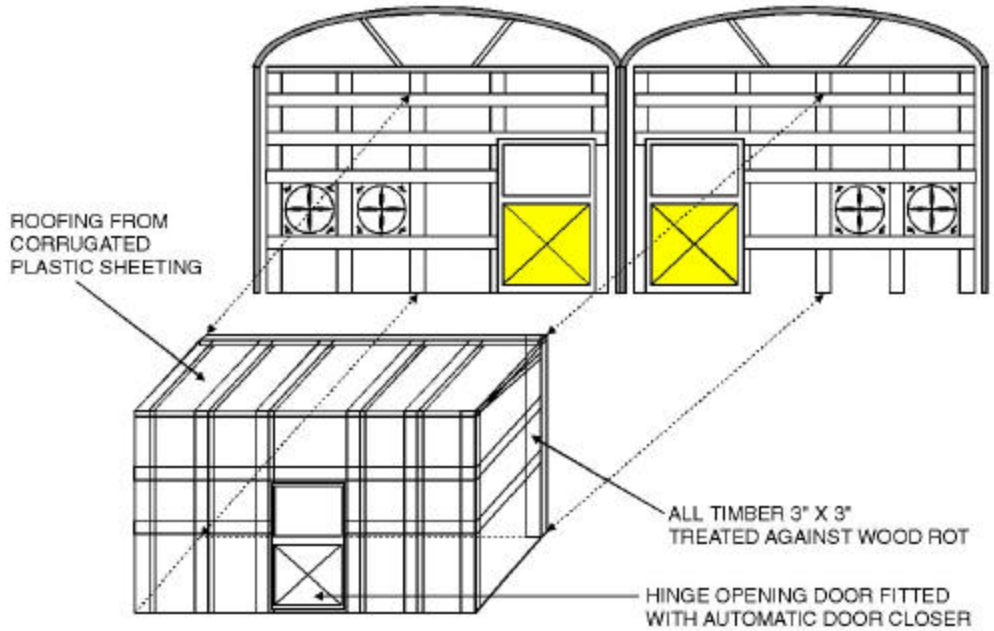


DOUBLE POLY SKIN ON ROOF AND ALL WALLS.
FANS AND BLOWERS FITTED WITH 40/40 MONO FOIL MESH NETTING
DOORS FITTED WITH AIR RETENTION BRUSHES
ENTRANCE THROUGH DOUBLE DOOR (AIRLOCK TYPE) SYSTEM

VIEW SHOWING MULTI-SPAN UNIT WITH COOLING PADS & FANS FITTED

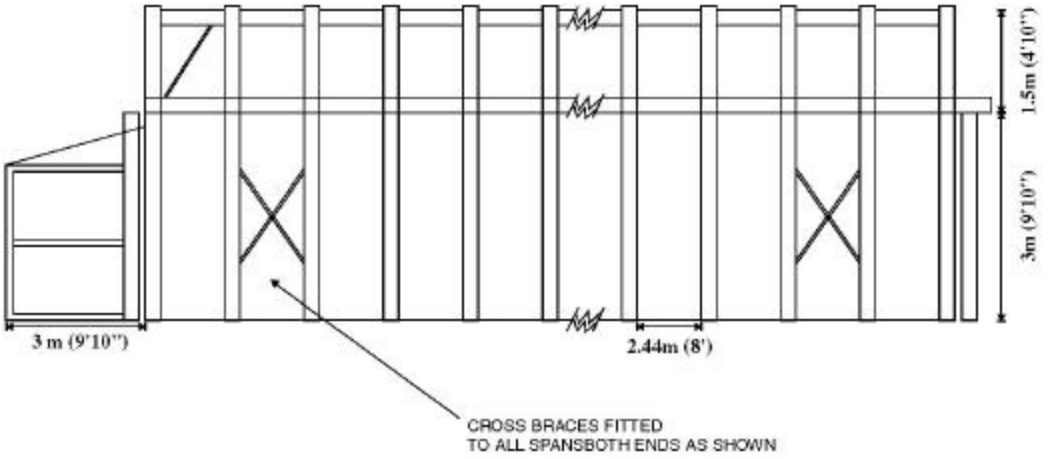


VIEW SHOWING DOUBLE DOOR ENTRY

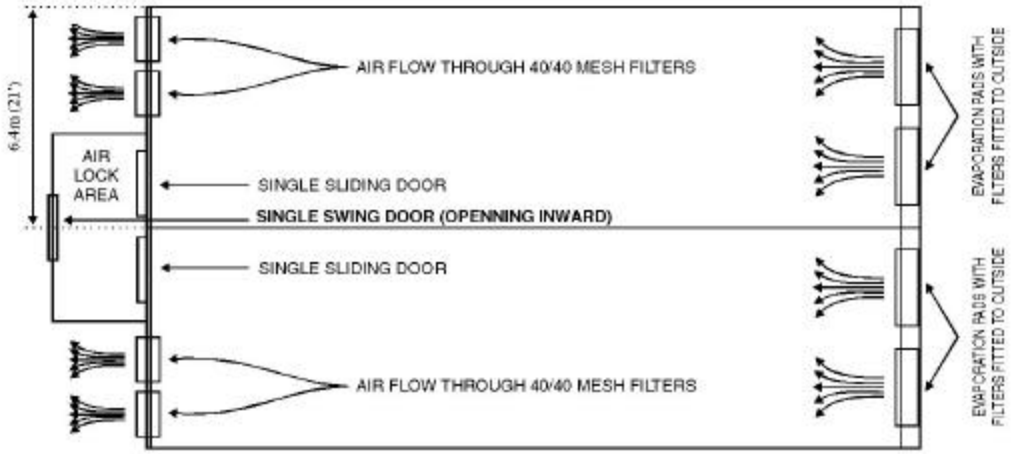


SINGLE DOOR EXTENSION UNIT

SIDE VIEW OF MULTI SPAN UNIT WITH DOUBLE DOOR ENTRY



PLAN VIEW OF GREEN HOUSE STRUCTURE



RECOMMENDED MINIMUM GROWING AREA 13m X 30m (2 X 6.4m SPANY 12 X 4.44m BAYS)

POTENTIAL PLANT CAPACITY - APPROX, 1,000 14" CLAY POTS, OR 2,000 LITRE POTS