Manual for Biosafety Level II Greenhouse for Research on Transgenic Plants at KARI Biotechnology Centre.

Murenga GM, Mugo SM, Odhiambo B, McLean S, and Taracha C

IRMA Project Document No. 14

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KARI/CIMMYT IRMA PROJECT

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Cover Photo: Biosafety Level II Greenhouse Complex at KARI Biotechnology Centre, NARL Kabete

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Participating Institutions

The Kenya Agricultural Research Institute (KARI) was established in 1979 with the express mission of increasing sustainable agricultural production by generating appropriate technologies through research, and disseminating these to the farming community. Inherent to this mission is the protection, conservation, and improvement of the basic resources, both natural and human. Such resources are critical for Kenya's agricultural development and expansion of the nation's scientific and technological capacity. KARI has an extensive history of productive collaborators with national and international institutes and universities, as well as with the private sector. (www.kari.org)

The Syngenta Foundation for Sustainable Agriculture provides major funding for the project. The Foundation is dedicated to fostering sustainable development in poor countries of the South through its support of programs and projects in the areas of sustainable agriculture, health, and social development. It is also an active player in development policy debate through its preparation and dissemination of research analysis. Further information about the Foundation may be found at its web site (www.syngentafoundation.com).

CIMMYT® (www.cimmyt.cgiar.org) is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center works with agricultural research institutions worldwide to improve the productivity, profitability, and sustainability of maize and wheat systems for poor farmers in developing countries. It is one of 16 similar centers supported by the Consultative Group on International Agricultural Research (CGIAR, www.cgiar.org). The CGIAR comprises about 60 partner countries, international and regional organizations, and private foundations. It is co-sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), the United Nations Development Program (UNDP), and the United Nations Environment Program (UNEP). Financial support for CIMMYT's research agenda also comes from many other sources, including foundations, development banks, and public and private agencies.

CIMMYT supports Future Harvest® a public awareness campaign that builds understanding about the importance of agricultural issues and international agricultural research. Future Harvest links respected research institutions, influential public figures, and leading agricultural scientists to underscore the wider social benefits of improved agriculture—peace, prosperity, environmental renewal, health, and the alleviation of human suffering (www.futureharvest.org).

The Insect Resistant Maize for Africa (IRMA) Project was launched is a collaborative effort between KARI and CIMMYT. Its primary goal is to increase maize production and food security for African farmers through the development and deployment of maize that offers resistance to destructive insect especially stem borers. To achieve this goal, project scientists will identify conventional and novel sources of resistance to stem borers and incorporate them into maize varieties that are both well adapted to Kenya's various agroecological zones and well-accepted by its farmers and consumers. Varieties and technologies that are appropriate for other African nations may be extended to them for their use.

Printed in Kenya.

The views expressed in this Biosafety level II Greenhouse Manual are those of the authors and do not necessarily reflect the views or policies of the respective institutions

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Manual for Biosafety Level II Greenhouse: For Research on Transgenic Plants

1.0 Introduction

The application of biotechnology and its wide range of tools pose new challenges to governments that require biosafety frameworks be in place for the safe and responsible use of the genetically modified organisms and their products.

However, there is a need for all stakeholders to be more serious about institutional learning, and change through a balanced approach. The focus should be on the most relevant and available scientific basis for the debate on biotechnology and its tools in addition to other life sciences contributing to the solutions to hunger and poverty in the world over.

Today, 'genetic engineering' is increasingly being applied in agricultural and horticultural research, health, environmental protection, industry and social sciences. However, 'fears' and 'concerns' raised about ethical and safety aspects have emerged that call for safe and responsible application of modern biotechnology to ensure avoidance of inadvertent harm to human health, environment and biodiversity. To ensure that these concerns are addressed biotechnology should be developed and applied within the framework of biosafety.

These frameworks should include putting in place appropriate biotechnology and biosafety legal provisions, regulations and guidelines related to specific use. To this end, the government of Kenya through the National Council for Science and Technology (NCST) developed and published the *Guidelines for Biosafety in Biotechnology* in 1998.

In the same breadth, the institutional Advisory Committee on Biosafety (KACB) developed a draft for KARI's *Guidelines and Regulations for Biosafety in Biotechnology Research* in 1994. These biosafety guidelines outline the general biosafety policies and procedures of the Kenya Agricultural Research Institute (KARI) to ensure the safety of research with [1]genetically engineered organisms, that of the products, the personnel, agriculture, environment and biodiversity.

[1]In this biosafety greenhouse manual, the terms "transgenic," "genetically engineered," and "genetically modified" are used interchangeably.

The methods for the safe handling of transgenic materials in both the biosafety greenhouse and/or laboratory environment are described in the *Guidelines for Biosafety in Biotechnology Research*. The general applicability of these guidelines on genetic modifications include all recombinant DNA (rDNA) methodologies.

Information about handling transgenic plants in biosafety greenhouses in Kenya is however, relatively sparse. Currently, though, there is no single source of practical guidance on managing biosafety greenhouses containing transgenics, nor on the requirements for building or renovating plant growth facilities to make them suitable for containing transgenic plants, the products and associated organisms.

KARI in conjunction with International Centre for Maize and Wheat Improvement (CIMMYT), with the support of Syngenta Foundation for Sustainable Agriculture, working on the Insect Resistant Maize for Africa (IRMA) project have developed a biosafety level II greenhouse complex (hereinafter referred to as biosafety greenhouse) at the Kenya Agricultural Research Institute's Biotechnology Centre. These facilities are new and operational.

The biosafety greenhouse contains three greenhouses, which meet the national biosafety standards based on the National Biosafety Committee (NBC) and the KARI Advisory Committee on Biosafety (KACB) requirements. These biosafety greenhouses at KARI Biotechnology Centre serve as bio-containment facilities that provide highly effective means of isolation and prevention of unintended transmission of genetic material. The disposal of genetic material from maize is prevented through reproductive, spatial and temporal isolation.

2.0 Audience

The primary users of this manual will include the biosafety officer, greenhouse managers, facility staff, research scientists and staff from all national regulatory entities that provide regulations and guidance for the safe release of plant materials including genetically modified organisms (GMOs) into the environment.

The biosafety officer and greenhouse managers, being responsible for the overall operations of a biosafety greenhouse facility, will benefit from a clear description of when, where, and why additional containment measures should be instituted, as well as practical guidance for managing the facility and personnel working in it. The biosafety greenhouse staff involved in the day-to-day care of transgenic organisms will gain and improve their understanding of the tasks involved when handling experimental materials that have been genetically engineered. Other persons working within the biosafety greenhouse facility (maintenance personnel, etc) will benefit from the basic knowledge of the purpose of containment, which will precipitate into safe handling of genetically engineered materials.

3.0 General biosafety greenhouse procedures and practices

This biosafety greenhouse manual is intended to be a simple and convenient reference on relevant and appropriate biosafety procedure for research on genetically engineered organisms and that of their the products conducted in the biosafety greenhouses. This manual is not all-inclusive but serves as a guide to all persons working in the biosafety greenhouse that houses transgenic materials.

3.0.1 Personal safety

3.0.1.1 Greenhouse coats

Greenhouse coats must be worn at all times and must not be removed from the biosafety greenhouse unless decontaminated.

- Dark blue greenhouse coats should be worn while working in general greenhouse area.
- Red greenhouse coats should strictly be worn while inside the biosafety greenhouses 1, 2 and 3.
- Red greenhouse coats must remain in the biosafety greenhouse unless while being washed or decontaminated.

Greenhouse coats will be provided to all biosafety greenhouse personnel and a limited number reserved for visitors. These coats should be kept in the designated cabinets.

3.0.1.2 Personal hygiene

- Hand washing must be done prior to exiting the biosafety greenhouse premises
- Greenhouse coats must be laundered regularly
- Greenhouse coats must be decontaminated before use.
- Eating, smoking and drinking and keeping of food and drinks are prohibited in the biosafety greenhouse facility.

3.0.1.3 House keeping

a) Remove all safety hazards and maintain good order in the biosafety greenhouse work areas.
b) All equipment should be inspected before use
c) All passages to the emergency exits must be clear at all times!
d) Wipe all bench tops and other surfaces in the biosafety greenhouse after each use with an appropriate cleaning agent and/or disinfectant.
e) All machinery under maintenance or adjustment should be tagged prior to servicing by only the authorized personnel.

f) All experimental transgenic materials must be sterilized and disposed appropriately in the designated biohazard bags and incinerated.
g) Unauthorized personnel (friends and relatives etc) must not be brought to the biosafety greenhouse but can watch activities from the visitors' platform.
h) Ensure that routine schedule for working over weekends and public holidays is strictly adhered to.

3.0.1.4 Fire prevention

Two fire extinguishers (carbon dioxide and water) and two emergency exits have been provided in the biosafety greenhouse facility, with an alarm system on each of the emergency doors. All biosafety greenhouse personnel must:

- Beware of the condition of the fire extinguishers and how to use them safely.
- Report any broken seals, damage, low gauge pressure or improper mounting to the greenhouse managers.
- Never use fire extinguishers unless you have been trained and gained confidence on their use.
- Be careful on the potential ignition sources such as electrical equipment (soil sterilizer, drill etc).
- Flammable liquids or chemicals must be stored in appropriate stores or safety cabinets.
- Make sure that all electrical cords are in good working condition. All electrical outlets should be grounded and should accommodate a 3-pronged plug.
- Never remove the grounding prong or use an adapter to bypass the grounding on an electrical cord.
- Never leave the electrical equipment working unattended for extended periods of time.

3.0.2 Personal protective equipment

3.0.2.1 Protective clothing

The greenhouse coats are designed to protect the clothing and skin against water, chemicals or otherwise that may spill. It should always be fitted to the personnel using it to cover upto the knee length.

These are special types of clothing made of fibre to protect the personnel against the hazardous effects of insecticides and fungicides during the spraying exercise or otherwise.

3.0.2.2 Respiratory protection

Certain biosafety procedure especially chemical applications can produce noxious fumes and/or contaminants; therefore respiratory protection is extremely necessary while working in such an environment.

Appropriate protective respiratory and body protective clothing must be worn at all times (rubber gloves, hand gloves, gumboots, respiratory masks etc) during insecticide or fungicide applications.

3.0.2.3 Hand protection

It is imperative to use protective hand gloves in the biosafety greenhouse while handling materials including transgenic seed (especially if it is coated with a preservative chemical) or chemical fertilizers. Before use, check to ensure the gloves (especially the latex gloves) are in good condition and free of perforations, punctures or tears.

When working with chemicals (such as fungicides or insecticides), wear thick hand gloves. Take precaution in checking for holes, perforations, punctures or tears.

Extreme care must be taken when removing the protective hand gloves. Peel the glove off the hand, starting at the wrist and working toward the fingers. Keep the working surface of the hand glove away from contact with your skin during removal. Used protective hand gloves, or contaminated disposable hand gloves should be discarded in designated containers (biohazard bags or waste containers).

3.0.2.4 Foot protection

Foot protection is meant to protect injury from corrosive chemicals, heavy objects, electric shock, and to give a better traction while walking on wet surfaces. Shoes (in these case gumboots) that completely cover and protect the feet are recommended. Appropriate closed-toed shoes or gumboots should be worn while working in the biosafety greenhouse.

The following shoe types that expose the feet in anyway must not be worn in the biosafety greenhouse; sandals, clogs, high heel shoes and any other

3.0.3 Biosafety greenhouse safety equipment

3.0.2.1 Fire safety equipment

Fire alarms are designed to alert by a loud and audible ‘ringing’ warning, all biosafety greenhouse personnel of the impending danger.

All biosafety greenhouse personnel are advised to be familiar with the exact location of the fire alarm station nearest in the biosafety greenhouse.

Fire extinguishers should be located in the passage way in biosafety greenhouse.

All biosafety greenhouse personnel are advised to carry out routine inspection of the fire extinguishers at least fortnightly. Any anomalies (broken seals, low pressure or any visible damage) observed should be reported to the greenhouse managers or the maintenance personnel for immediate replacement or action.

3.0.2.2 Seed storage

This is the most sensitive area, where restriction should be observed, particularly when handling transgenic seed. Storage of transgenic seed should be done strictly in the designated seed storage cabinets and always under lock and key. **Strictly** all transgenic seed should be treated with a seed preservative, and kept in appropriately labeled envelopes and stored in the designated seed storage cabinets. A seed inventory must be kept so as to monitor the use of the transgenic seed.

3.0.3.3 General storage

This store should be used for handling other supplies to be used in the biosafety greenhouse only. It should always be under lock and key. The biosafety greenhouse staff are advised to keep an inventory of items, supplies or otherwise to monitor movement and use of materials in these store.

3.0.4 Safety of biosafety greenhouse equipment

3.0.4.1 Heating devices

Electrical devices that supply heat for soil and plant material sterilization shall commonly be used in the biosafety greenhouse. The electric devices are the soil sterilizer and an autoclave.

Improper use may result in fire, burns or explosions to the user or otherwise. All biosafety greenhouse personnel are advised to closely monitor the devices under use ***all the time!***

Before using any heating device all personnel are advised to:

- Check if the unit has an automatic shut-off switch in case of overheating.
- Note the condition of the electrical cords and have the replaced as may be necessary.
- Ensure that the device is maintained as required by the manufacturer.

All biosafety greenhouse personnel are advised to always check all heating units in use and turn off those without automatic shut-off before leaving the biosafety greenhouse for any extended period.

3.0.4.2 Other electrical equipment

Care must be taken when handling these devices. The biosafety greenhouse personnel using any of the equipment above should be knowledgeable enough and confident to avoid accidents. These include vacuum cleaners, electrical drill and other equipment.

4.0 First aid and emergency procedures

First aid and emergency procedures are life saving. Every biosafety greenhouse personnel is advised to be familiar with information described below so as to curtail any dangers or disasters that may arise. It is the responsibility of the personnel to report any bodily harm or injury or damage on property to the biosafety officer or the greenhouse managers, failure to which may lead to dismissal.

Ensure that all names and numbers of the biosafety officer and the greenhouse managers to be contacted in case of an emergency are clearly posted on each door of the biosafety greenhouse and outside the main entrance door of the biosafety greenhouse.

Ensure that you are familiar with the location of the safety devices below:

a) First aid kit
b) Fire extinguisher
c) Fire alarm
d) Protective clothing for insecticide/fungicide applications
e) Protective respiratory gear

In case of fire and/or an explosion;

- Activate the fire alarm system,
- Call the fire department to report the emergency,
- Attack the fire if possible using appliances provided (only if you are knowledgeable enough on their use),
- Move to the fire assembly point.

A fire action sign has been posted to indicate the necessary steps to be undertaken in case of a fire outbreak.

All passages to the safety equipment and to emergency doors must be clear at all times! Report immediately any damage or breakage on windows or seals to the biosafety officer or greenhouse managers. Cover the broken glass window using a plastic paper and fasten it with an adhesive. Note that covering should start with the outer side of the window.

The same applies to damage on any other equipment or item used in the biosafety greenhouse.

4.0.1 Wounds, minor cuts and bruises

- Place a sterile pad over wound, minor cut or bruise and apply direct pressure evenly with the opposite hand.
- If bleeding persists, raise the area above the level of the heart.

- Cleanse the area with an appropriate disinfectant, soap and/or clean water.

4.0.2 Significant bleeding

- Place a sterile pad over wound, minor cut or bruise and apply direct pressure evenly with the opposite hand.
- If bleeding persists, raise the area above the level of the heart.

- Cleanse the area with an appropriate disinfectant, soap and clean water.
- Otherwise take the injured victim to the nearest hospital for medical attention.

4.0.3 Thermal burns

This may be due to sunburn or mild steam burn. Discoloration or redness of the skin mild swelling and pain are the characteristics of first-degree burns. The first aid procedures for first degree burns are as follows;

- Apply cold water or immerse the burned surfaces in cold water for at least 10-15 minutes.
- Take the injured victim to the nearest hospital for medical attention.

Second and third degree burns are characterized by red or mottled skin with blister formations and white charred skin respectively. The first aid procedures for the second and third degree burns are;

- Cover the burned area with a clean dry piece of cloth.
- Call an ambulance immediately or otherwise take the injured victim to the nearest hospital for medical attention.

4.0.4 Chemical burns

- If hazardous chemicals especially insecticides or fungicides come into contact with the skin, the personnel affected should be unclothed, modesty not withstanding and all the shoes removed. The affected areas should be rinsed with large quantities of water for at least 15-20 minutes.

- If hazardous chemicals, insecticides or fungicides or otherwise come into contact with the eyes, the area of the eye should be rinsed thoroughly with large quantities of clean lukewarm water for at least 15 minutes.

4.0.5 Inhalation of chemical substances

Pesticide or spaying of chemicals in the biosafety greenhouse shall be undertaken on Fridays depending on the pest regime and incidence. A plaque indicating; '*Danger! Do Not Enter. This Room Has Been Sprayed With Insecticide*' should be hanged clearly at the entrance of the respective biosafety greenhouse. This warns all personnel involved not to enter and expose themselves to the dangers of inhalation of fumes resulting from this exercise.

- In cases of accidental exposure the victim should be evacuated to an area with fresh air outside the biosafety greenhouse facility.
- Otherwise take the injured victim to the nearest hospital for medical attention, and take note of the name and type of chemical, insecticide or fungicide that may have caused this accident.

4.0.6 First aid kits

First aid kits that meet the national biosafety standards for equipment in every biosafety greenhouse (s) are recommended for use. The first aid kit should contain a variety of items specially selected to carry out emergency treatments of cuts, burns, eye injuries, or sudden illnesses.

Each item in the first aid kit should be individually packaged and its contents checked weekly to ensure that expired items are disposed and replaced or otherwise replaced.

The biosafety officer and greenhouse managers are solely responsible for the maintaining the contents of the first aid kits. No oral medication should be dispensed from the first aid kit.

4.0.7 Labeling and use of labels

Labels should be the basic, initial source of information to all biosafety greenhouse personnel when working in the biosafety greenhouse, while handling transgenic seed, or hazardous chemical substances (including insecticides or fungicides) or otherwise. All containers of the hazardous chemical substances should include the name of the chemical, appropriate hazard warnings, name and address of manufacturer and the antidote or otherwise the responsible party.

The biosafety officer and greenhouse managers should ensure that all in-coming chemicals, insecticides or fungicides bear all a label specifying the name of the hazardous chemical, appropriate hazard warning, name and address of the manufacturer or the responsible party.
All biosafety greenhouse personnel are advised not to deface labels on containers of the chemicals

All biosafety greenhouse personnel are advised to label appropriately the secondary containers in cases of transfer of chemicals from the manufacturer's original container. This would help in chemical identity and the corresponding hazard warning.

4.0.8 Hazard information

Hazard warnings may be in form of pictures symbols and words or a combination of the later, which convey the potential hazards of the chemical, in question.

Picture hazard warnings are conspicuous and they help to identify properties and class of the chemical as biohazard, explosive, poison, flammable corrosive etc.

Symbol hazard warnings provide basic information that determine the precautionary measures that should be undertaken when handling the chemical, transgenic seed or when dealing with fire.
An example is ' biohazard symbol and sign posted on the main entrance door and all entrances to the biosafety greenhouses 1, 2 and 3 or 'transgenic seed' on every envelope that contains these seed.

Word warnings are intended to capture the workers immediate attention. For example; 'No Smoking, No Eating, No Drinking', 'Fire Exit Keep Clear!', Flammable, Poison etc.
These word labels should be written in English but other languages may be used as deemed necessary.

Sign words are warnings used to designate the degree of hazard. For example 'Danger! Do Not Enter This Room Has Been Sprayed With Insecticide'.

5.0 Personnel orientation, information and training

For responsible and safe conduct in the biosafety greenhouse all personnel intended to work in the biosafety greenhouse must be introduced to the new code of practice and to institutional biosafety guidelines.

The basic training will include biosafety greenhouse operations, general information on handling of transgenic materials, biosafety, containment procedures, practical applications, procedures and operations, management of target organisms, and the regulations guiding the management of biosafety greenhouses.

All personnel intended to work in the biosafety greenhouse must know the purpose of containment, how it is attained and the facilities and practices that ensure compliance with the established biosafety guidelines and regulations.

The biosafety officer and the greenhouse managers will train and inform the personnel on the necessary precautions and requirements in the management of the genetically engineered materials in the biosafety greenhouse. Continuous in-service training in safety measures at the biosafety greenhouse will be provided.

Before working in the biosafety greenhouse area all personnel will be required to be familiar with the following:

(a) All the biosafety procedures in the biosafety greenhouses including emergency procedures, safe handling, biological inactivation and disposal of experimental materials
(b) All the biosafety procedures that may lead to the loss of bio-containment, unintended release of pollen.
(c) All the biosafety protective measures such as appropriate work practices, emergency procedures and protective clothing
(d) All the biosafety procedures involving the security and a bio-containment logbook in which every person coming into or out of the biosafety greenhouse must sign.
(e) All the biosafety procedures involving record keeping of all on-going-experimental work.
(f) Record keeping of inventory of all equipment, tools, supplies and any other relevant information as may be deemed necessary.

5.0.1 Restrictions and regulations to the biosafety level II greenhouse complex

The following restrictions and regulations will apply to the biosafety level II greenhouse complex at Kenya Agricultural Research Institute (KARI) at Biotechnology Centre:

a) Unauthorized personnel are not allowed entry
b) Titles of all experiments must be posted at entrances
c) Doors must be locked at all times
d) Smoking is prohibited
e) Drinking and eating are prohibited
f) Greenhouse coats must be worn at all times and must not be removed unless decontaminated
g) Hand washing is required prior to exiting the biosafety greenhouse
h) Pollen release of genetically engineered materials must be restricted
i) Use of [2]exotic organisms is prohibited
j) Work implements, equipment and tools must not be removed unless decontaminated
k) Removal of plant materials is restricted and must meet stated guidelines

The above restrictions and regulations must be adhered to ensure the safety of research with genetically engineered organisms, that of the products, the personnel, agriculture and the environment.

[2] An exotic organism refers to any microorganism or pathogens (including viruses, viriods, and mycoplasms) not naturally present in the region.

6.0 Preliminary set-up procedures for the biosafety greenhouse

6.0.1 Soil mix

The ideal soil mix used in the biosafety greenhouse is a mixture of three parts of alluvial soil to one part of sand and one part of peat moss. We are using one wheelbarrow of soil to one wheelbarrow of sand to one bale of coconut peat, which are mixed well to obtain this ratio of 1:1:1:1.

6.0.2 Fertilizer types and rates of application

Fertilizer should be applied after a period of every twenty days from the transplanting. To the large pots, 2.5 grams of urea/triple super phosphate (by volume) are added in the ratio of 2:1. For medium pots, add 1.25 grams of urea/triple super phosphate (by volume) in the ratio of 2:1. Soluble NPK (19:19:19 + MgO) fertilizer (2g/L of water) is applied at 500ml per large pot and 250ml for medium pots.

Putative transgenic seed should be planted and the germinating plants acclimatized to the biosafety greenhouse environment, watering with the soluble fertilizer NPK above at 250ml for a tray of plants at the rate of 1g/L of water.

6.0.3 Pots

Four types of durable plastic growing pots will be used.

- Large pots (12-14 inches in diameter) for growing plants to maturity,
- Medium pots (6-8 inches in diameter) for plant evaluations and later transplant to the large pots .
- Small pots (3-4 inches in diameter) for quick evaluations of large numbers of genotypes or lines, and
- Small transfer pots (1-2 inches in diameter) for the transferring putative transgenic from growth media.

All pots should have drainage capabilities (perforations at the bottom) enough to avoid accumulation of water.

6.0.4 Watering

Watering is an essential component for the growth of plants. Therefore plants should be checked daily to ensure enough supply of water. At all times the soil mix should be moist to feel not too dry or too wet in all pots. Smaller pots should be watered more often than the others. Watering of plants should be done at mid-morning and in the late afternoon.

The decision to water the plants or otherwise depends solely on the personnel working in the biosafety greenhouse. *A useful rule of thumb is to test each and every pot with your fingers*.

6.0.5 Lighting and temperature

Different plants have different lighting and temperature requirements. Ideal temperatures for maize are 25-35^{o}C, with the optimal temperatures at 28^{o}C. Adequate light intensity and quality is essential for the growth of plants. The KARI biosafety greenhouse is located within the tropical environment with no major variations in both temperature and light, therefore no special light requirements may be necessary unless work will be done on photosensitive genotypes in the future.

Every morning, recording of maximum and minimum temperatures and relative humidity should be done in the biosafety greenhouse (house 1, 2 and 3) to provide data on water requirements and, also monitor and provide ideal conditions necessary for plant growth.

The average range of temperature conditions in the biosafety greenhouse for the given period should be recorded. High temperatures are prohibitive to pollen production, therefore, watering the floor should be done to increase the relative humidity and reduce the temperatures to ideal conditions. The evaporative cooling systems have been fitted on all the greenhouses to improve on airflow within. Routine maintenance checks on all evaporative coolers should be done.

6.0.6 Staking

Staking must be done at least twenty days after transplanting the plants that have been found to have insect resistance and the desired phenotypic characteristics. The practice should be undertaken routinely to avoid lodging during watering or movement of the personnel within.

6.0.7 Washing

Sodium hypochlorite and phenolic compounds are the disinfectants recommended for general biosafety greenhouse use. Ensure that each disinfectant used has been validated for its indicated use in the biosafety greenhouse

All pots, trays and other equipment used in handling of transgenic material must be decontaminated. The pots should be washed using water mixed with soap and a commercial bleaching agent (sodium hypochlorite solution).

The bleaching agent helps in disinfecting the pots, trays etc by desiccating the insect eggs. After washing the pots, equipment, tools etc should be air dried on the sun within the biosafety greenhouse washing area for a few hours.

6.0.8 Insect and disease control

The most common insects in the biosafety greenhouses are the spider mite, white flies and aphids. The application of commercial fungicides and insecticides should suffice for the control of common insects and diseases. Commonly Ridomil (5ml/10L of water), Methomex 90 SP (400-500g/ha), Talstar (3ml/10L of water), Folicur EW 250 (200ml-750ml/ha, Malathion, Pirimor and others will be used.

6.0.9 Incineration and disposal of materials

Waste is anything to be discarded. All plant materials from the pots in which plants had been growing in the biosafety greenhouse, including material from either selection, de-tasseling etc must be chopped into small pieces placed into autoclavable plastic bags (*Bio-hazard Autoclave Disposable Bag")* and steam-sterilized for at least three to four hours at 80-100^{o}C.

The materials are then incinerated at a designated area outside the biosafety greenhouse and disposed in a trench within the biosafety greenhouse compound. The soil mix should be sterilized by steam and then stored at an open site to dry before recycling.

7.0 Security and safety of research

Security is the most critical of all the issues. Safety of research using genetically engineered organisms, the products, the personnel, agriculture and the environment and biodiversity are paramount. There are practices and precautions that every person involved in any activity related to this **must adhere to at all times**!

Containment is required for movement in and out of the biosafety greenhouse i.e. a bio-containment logbook, in which every person coming into or out of the biosafety greenhouse must sign, biologically inactivate experimental organisms at the end of experiment and decontaminate equipment, and supplies, appropriate caging and precautions for dispersal of pollen and control of motile insects.

Clear precautions and practices should be posted on the doors of every biosafety greenhouse; personnel must read and follow instructions, a biosafety greenhouse manual is available to advise of consequences or contingency plans.

The international biohazard warning symbol and sign is displayed on the biosafety greenhouse access doors and also an indication on the special requirements for entry into the area as indicated above.

8.0 Screening putative transgenic maize plants

The putative transgenic maize plantlets received from the biosafety laboratory should be placed under shade conditions for three to four days for the plants to acclimate to the biosafety greenhouse growth conditions. The plantlets should then be transplanted from small transfer pots (1-2inches in diameter) into medium pots (6 inches in diameter) under sunlight conditions and grown for another 2-3 weeks. During this stage, the plants should be fertilized with Soluble NPK (19:19:19 + MgO) fertilizer. Further screening should be undertaken as indicated subsequently.

All the maize seedlings should be colour coded with stakes and each specific coloured stake should undergo different screening, subject to the advise by the biosafety officer and the greenhouse managers.

The screening of putative transgenic maize plants should be accomplished through various methods. These methods include direct infestation with first instar larvae of African stem borer.

8.0.1 Direct insect infestation on plants

Direct insect infestation on maize plantlets is another method used for screening putative transgenic plants containing the genes and promoters. Before insect infestation, plants should be inverted to remove any accumulated water in the whorl, 15-20 first instar larvae of African stem borer (*Chilo partellus or Busseola fusca)* should be placed in the maize whorl at the 6-8-leaf stage.

Feeding damage will be monitored daily to ascertain that larvae are alive. The expected observations should be that susceptible plants (non-transgenic) should show significant feeding damage on the leaves while the resistant plants may exhibit small holes resulting from the insect feeding and dying later due to *Bt* endotoxin. The responses of the insect infestation on plants should be made within one to one and half weeks after infestation.

Other stem borer species that will be used in insect bioassays on include *Chilo orichalcocillieulus, Eldana saccharina, and Sesamia calamistis*.

9.0 Pollination of transgenic plants

Before pollination, plants should be observed closely, and only healthy looking plants should be self or cross-pollinated. Everyday, all plants should be checked and glycine bags are used to cover the ear before the silks appear. Pollinations of transgenic maize will be made only in the biosafety greenhouse, and only red-labeled pollinating bags will be used to show that it is transgenic seed.

The step-wise considerations that should be followed during the self or cross-pollination of transgenic plants at the biosafety greenhouse are as indicated below:

9.0.1 Ear shoot bagging

It is one fundamental requirement of all the pollinating operations, it is the most difficult to do properly. It is more critical in the biosafety greenhouse, that the ear shoots (usually 6-7 leaf from the cap) be covered using glycine bags before the silks (styles) emerge in order to protect the silks from being contaminated by pollen from other events until the desired pollen can be applied.

Shoot bagging should be a daily operation no sooner than the first tassels appear. At this time the tip of the first ear shoot is visible in the axil of the 6th or 7th leaf down from the top of the plant. The shoot should be covered by placing the bag over the tip of the shoot with the longer lip of the bag next to the stem and so that the shorter lip of the bag slides between the tip of the ear shoot and the adjacent leaf sheath.

The bag should be given a sharp downward pull to firmly attach it between the stalk and the ear shoot. The plants should be examined every day to cover new ears as they emerge. At this time those bags already on the plants may be pulled down again to make them more secure.

Large ears or leafy tips may grow through and push the bag out from its original firm position. In special cases the leafy part should be trimmed using scissors and a bag replaced. Other less vigorous plants may be observed to produce silks while the ear is still completely hidden in the sheath.

In such cases, pull back the sheath slightly and bend the leaf down, or rip the leaf off at the collar (of the leaf), so the shoot bag will fit securely over the ear and the leaf sheath surrounding it. Under natural conditions receptive silks appear over a 7-8 day period. Ideally, only the top ear is enough, but it is recommended to cover both just in case the first ear does not develop good silks.

The timing of bagging is important; bagging too early is not very satisfactory because the tissues will not hold the bag firmly in place while late bagging risks exposure of silks and resulting contamination. Never should ears be bagged after silks appear. It is important that the persons working in the biosafety greenhouse be honest, otherwise contamination will obviate the expected results of the experiments.

9.0.2 Cutting back an ear

It involves cutting off, using a scissor or sharp knife, the tip of the husks and leafy tissue ear with first silks visible and then re-covering the ear to prevent exposure and possible contamination.

Cut squarely and cleanly across the ear and as far down the husks as possible, without cutting off the tip of the cob inside. The shoot bag covering the cut silks should be labeled to indicate the cut back ears. After a period of 24 hours silks should be observed to form brush like silks and should then be pollinated. Pollen applied at this time reaches all silks resulting in a full-fertilized ear.

9.0.3 Tassel bagging for pollen collection and pollination

For controlled pollination it is necessary to collect viable pollen. The tassel should be covered with the bag (brown paper tassel bag) keeping the tassel as flat (horizontal) as possible.

Pull the bag down past the first flag leaf (which may be removed) then fold the base of the bag firmly around the sheath and stem of the tassel and finally secure it in place with a regular paperclip.

To collect the pollen, carefully bend the plant so that the open end of the bag is higher than the closed end. Shake the bag and tassel sharply. Withdraw the tassel while taking care not to allow the open end of the bag to be low enough so that the pollen falls out of the bag. The pollen can be carried in this manner to make a self-pollination or to cross on silks of one or two nearby plants. The environmental conditions determine the viability of the pollen; therefore pollination exercise should be carried out as quickly as possible.

Pollination begins as soon as tassels are seen to be shedding pollen. If tassels are bagged in the morning sufficient time of at least an hour and half should be allowed for all stray pollen grains to die and for anthers with fresh pollen to emerge inside the bag. Depending on the temperature and humidity conditions, pollination should be accomplished from 11.00am to 2.00pm for the best results. Also, sunny conditions are a must as cloudy weather decreases amount of pollen produced for the day.

9.0.4 Dating of tassel bags

Dating of tassel bags is an important aspect to enable monitor efficiency of pollination. The date of pollination should be recorded on all tassel bags. With the closed end of a tassel bag as the top, starting at

the left margin and finishing at the right margin, using a permanent marker write the event number of the female parent by the event number of the male parent. If it is self-pollination indicate the event number and mark **X** and encircle it.

9.0.5 Seed management

Pollination should aim at having two plants pollinated well to produce enough quantities of seed. Following the pollination exercise, the plants should be de-tasseled. Each plant that grows to maturity should be allowed to dry until all seeds show a black layer and are dry.

At times, the husks should be open to speed up the process of drying down. Seeds from each ear should be shelled by hand and viable seed treated with insecticide and retained. Seed viability tests will be undertaken using the established standards methods.

The seed from each ear should be placed in an envelope and labeled with the origin, crossing, and quantity of seed from the respective ear. An inventory of the seed must be kept. It is strictly important that the transgenic seeds of maize be kept under lock and key in the designated seed storage cabinet.

10.0 Addresses of participating institutions

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2. International Centre for Maize and Wheat Improvement (CIMMYT),
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3. Syngenta Foundation for Sustainable Agriculture
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11.0 Glossary.

- **Biotechnology.** The tools and technology that are used to make products from biological systems (cheese making), to carry out processes using biological substances (enzyme-based processing such as wine making), or to modify biological systems in order to improve performance or produce materials (breeding, tissue culture, cloning, and transgenics)

- **Biosafety.** The safe application of biotechnology and pathology and the policies and procedures adopted to ensure this.

- ***Bt, Bt* endotoxins or *Bt* proteins.** Crystalline compounds produced by bacteria *Bacillus thuringiensis* that are toxic to selected insect orders: *lepidoptera, diptera and coleoptera.* Genes encoding Bt toxins have been transferred to plants to confer protection from insects. Bt toxins are not harmful to humans.

- **DNA (deoxyribonucleic acid).** The double stranded molecule that encodes genetic information. It is made up of four different kinds of bases, which are abbreviated A, C, T and G.

- **Exotic organisms.** Any microorganism or pathogens (including viruses, viriods, and mycoplasms) not naturally present in the region.

- **Endotoxin.** A complex bacterial toxin composed of protein, lipid and polysaccharide, which is released only when the cell opens to release cell contents.

- **Genetic engineering.** The technique of removing, modifying or adding genes to a DNA molecule in order to change the information it contains. By changing this information, genetic engineering (modern biotechnology) changes the type or amount of proteins an organism is capable of producing.

- **Genetically engineered/Genetically modified or transgenic material.** Any living organism whose genome organization has been modified by the addition of gene(s) from other organisms (natural DNA sources) or from DNA synthesized in the laboratory using technologies such as bioengineering or recombinant DNA.

- **Transgenic**. Plant or animal material whose genetic hereditary DNA has been transformed through the addition of DNA from a source outside its normal gene pool, using recombinant DNA techniques.

- **Herbicide resistance (tolerance).** The inherent ability of a plant to survive and reproduce following an exposure to a dose of herbicide that would normally be lethal to the target plants.

- **Instar.** Any of the life stages of an insect or arthropod

- **Recombinant DNA.** DNA farmed by combining segments of DNA from different organisms

- **Recombinant DNA technology.** Procedure used to cut and join together DNA fragments in a cell free system (an environment outside the cell or organism)

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