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Knowledge Creation

PROCEDURES FOR SAFE WORKING WITH GENETICALLY MODIFIED (GM) PLANTS

INSTITUTO DE TECNOLOGIA QUÍMICA E BIOLÓGICA



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1. INTRODUCTION

For work with GM plants, only the risks posed to human health are covered by the Contained Use Regulations. However, the potential for damage to the environment must be assessed under the requirements of the Environmental Protection Act and the Genetically Modified Organisms Regulations. The objective of the risk assessment is to determine the likelihood and the possible consequences of an accidental release of a GMO from containment into the environment. In a properly maintained and managed facility with the correct containment measures in place, the likelihood of such a release will be low. Nevertheless, it is important to identify all possible hazards and consider any routes by which the GMO could be released (including waste disposal, equipment failure and spread by humans or vermin).

To accomplish this claim and to support researchers work, recommendations from official Health and Protection Agencies were compiled in order to give guidance for containment, housing, environmental/personal safety and standard operative proceedings applied to GM plants manipulation.

ITQB as official permission to carry out research with microorganisms and genetically modified organisms of biohazard level 2, general safety measures and "Good Microbiological Practices" are described in the "SAFETY AND HEALTH AT WORK" manual. Although GM plants studied at ITQB belong to biohazard level 1 (BL1), there was the need to buildup a greenhouse with contained use characteristics to prevent transfer of GM material into the environment. Therefore, the new facility for housing GM plants demanded adding special protocols not considered in the Safety Manual and described herein.

2. DESCRIPTION OF THE FACILITY

The facility is located inside ITQB greenhouse park lot (draw in green) and has restricted controlled access.



In order to prevent ingress by vermin, birds or insects that could disseminate plant parts, pollen or seeds, the contained manipulation of GM plants greenhouse is preceded by an anteroom, and is equipped with ventilation, temperature and light control, tight lock doors, a running water point, no drains or sewer, sealed infrastructural support and HEPA filters in the ventilation openings. The walls, floors and working surfaces are finished with non-absorbing materials and are easy to clean.

Research equipment and accessories are dedicated to the facility and cannot be freely removed (see sections 5.4 and 6).

Anteroom entrance has the appropriate signs indicating "restricted entrance", "keep door closed" and "responsible person contact"; greenhouse entrance has signs indicating biohazard level, "keep door closed" and "emergency contacts".

3. ACCESS TO THE FACILITY

Work with and access to the GM plants greenhouse is granted by the Principal Investigator (PI) responsible for research being developed inside the facility. A signed list with authorized names is appended to the **Access logbook** kept at the Safety Office (1st floor). The Access logbook must contain information about who is entering the GM plants facility (legible names, data and time). Candidates to gain access to containment greenhouse must own an ITQB ID card and internal training certification on GM plants manipulation (have read this manual and passed the test). Anteroom and greenhouse keys will be delivered after signing the Access logbook and must be returned immediately after exiting the facility.

Sporadic visitors (e.g. auditors) or maintenance staff can only enter the facility with the responsible PI ensuring all containments rules were followed out.

4. SAMPLES AND WASTE MATERIAL TRACKING

All samples (plants or seeds) must be labeled with a unique tag and registered in the **Sample logbook**. The Sample logbook must contain information about sample type, ID, amount (if applicable), activity to be performed (storage at fridge or freezer, to grow, to be analyzed at the lab, waste for autoclave or incineration, etc.) and the researcher signature.

Is PI obligation to verify all entries in the **Sample logbook**, signing the pages, and to perform a security copy once a month, kept in PI archives.

5. WORKING AT THE FACILITY

Primary physical containment is provided by both the facility and the containment equipment within the facility. Containment is maintained through the good laboratory practices of staff to maintain the physical conditions required for containment and who are trained to notice when conditions are not normal and take swift action.

5.1 Entering into restricted area

Routine access to facilities housing confined research material is restricted, regardless of the biosafety level. Such restrictions are intended to minimize the spread of pollen, seed, or other propagative material that could be carried by people moving between rooms or facilities. Thus, all researchers and support staff working at the GM plants greenhouse are required to take precautions in order to prevent any transfer of GM material to the environment. So, into the anteroom 2 closets can be found: one with protection clothing (lab coat, shoes cover and hair cap, and gloves) to be used inside the greenhouse and the other to keep personal belongings while working at the facility. Hence, after entering the anteroom, all personnel are requested to dress up by the following order:

1. lab coat
2. hair cap

3. shoes cover

4. gloves

Long sleeves lab coat should be worn closed and wearing jewelry or fashion accessories are highly undesirable. Once fully equipped, the greenhouse can be open and the researcher can proceed with work. Stepping in and out of the greenhouse should be avoided. If some work must be done in the anteroom, shoes cover should be changed before re-entering in the greenhouse. Anteroom and greenhouse **doors** must be **kept closed** as much time as possible and, if more than one person is working inside, **doors can never be open at the same time**.

Protection clothing cannot be worn outside the facility. All items are obligatory removed and kept into the anteroom in the appropriate place (cabinet or waste bin).

5.2 Samples, accessories and lab material

All samples, accessories and lab material entering or exiting the GM plants facility must be transported inside sealed packages or tight close sanitized boxes. To prevent the infestation by cardboard pests, storage boxes are not allowed inside the facility and must be replaced by plastic or metallic autoclavable boxes. Minimal amount of stocks should be kept into the anteroom including freshly prepared disinfectant solutions.

Soil, lab ware and tools must be sanitized (by autoclave or chemical disinfection) before and after being use at the facility.

5.3 Sample ID system and handling

a) Sample identification

When first obtained or received from external sources, samples have to be labeled and registered in **Sample logbook**. Sample ID should be short and include relevant information for unequivocal fast sample identification (e.g. SDS-SCE1a-1, where "SDS" is an acronym for project name "Sumo Drought Stress", "SCE1a" is the name of the gene modified or introduced, and "1" in the line number). For new generations arising from previous ones, ".number" is added to previous sample ID (e.g. "SDS-SCE1a-1.1"). Alternatively, if confidentiality rules apply to sample name, gene name can be omitted (e.g. "SDS-1.1"). Independently of which sample ID is chosen, a detailed description of the line should be entered in the **Sample logbook**,

whenever the sample is registered for the first time. If any biological material, including plants and seeds, is found to have been misidentified, it should be immediately considered as biological waste and be treated as such.

b) Sample handling

Samples should be labeled at all time. Seed envelopes have to be individually labeled and closed at all times, unless seeds need to be transferred elsewhere (e.g. for sowing). Only ONE seed envelope can be opened per time. Plants have to be labeled individually. Plant ID should include the sample line ID (see section a) above), plus the plant number (e.g. SDS-SCE1a-1 #3, where "#3" is plant number).

5.4 Hygiene and safe working procedures

To minimize the risk of spill, all work must be conducted and kept inside secondary containment trays including seeded soil trays. All spills should be cleaned immediately by sweeping or absorbing with paper and sanitized with the appropriate disinfectant solutions (bleach, 70% ethanol, commercial solutions, etc.). Tight close autoclave containers are available to transport liquid or solid waste, lab ware and accessories.

Solid waste is separated in 2 categories depending on the sanitization treatment to be applied. Disposable lab ware, soil, paper towels and discarded protection clothing must be trashed into autoclave plastic bags. Plants, seeds or other GM contaminated materials must be placed into appropriate incineration bags. Bags are kept into properly labeled closed bins until being removed. Autoclave decontamination is available on site (ITQB main building); incineration is available off site (contract with specialized waste management company).

Solutions, excess run-off water from watering pots and sink drain waste water are collected into special 10 L autoclave containers, and are autoclaved prior to being discharged.

Lab equipment or environmental control apparatus must be cleaned or decontaminated prior to be removed from the facility, repaired or serviced; this includes blown tube lamps, maintenance parts and accessories being replaced.

6. CLEANING AND DISINFECTION

The facility (surfaces and permanent construction) should be easy to clean and maintained in a tidy condition; no cracks or gaps should be present. All cleaning procedures must be done by facility staff.

Therefore, to prevent the survival of organisms unintentionally transported outside the greenhouse environment, experimental materials must be rendered biologically inactive (devitalized) before disposal and facility environment should be kept the most cleanly and decontaminated as possible. Special precautions must be taken to avoid pollen dissemination, if plants are intended to grow till full mature state, by securing a glaze or fine mesh bag around the flowering stems.

Prior to start using the greenhouse, and whenever necessary, the exposed surfaces should be sanitized. It is recommended to apply vaporized hydrogen peroxide, as there is no toxic residue, or similar chemical disinfectants.

Clean periodically all growing area surfaces with standard cleaning solutions (e.g. sodium hypochlorite – house bleach) or plain soap and water. Support bench must be decontaminated with bleach solution or 70% ethanol at the end of the work. Material from experiments should be inactivated by chemical disinfection or autoclaving containers, potting media and lab ware including storage boxes and trash bins. The recommendation is to autoclave materials at 1.2 bar pressure and 121 °C for 15 – 180 minutes, depending on the type and state of the material being sterilized. Remaining GM materials (plants, seeds, etc.) should be incinerated to ensure complete destruction.

Cleaning material (mop, cleaning cloth, vroom brush, etc.) and lab coats have to be sterilized by autoclave before being washed. Storage of paper towels should be avoided inside the facility; if possible leave surfaces drying by airing after decontamination.

7. EMERGENCY PLAN

The facility is connected with ITQB main building by a mobile phone line. A first aid box, with material to treat small accidents, is available and located inside the protection clothing cabinet. A list of emergency phone numbers, including the contacts from ITQB team with first aid training, is posted near the phone. In case of emergency:

1. Immediately call for help and assistance. Unless the situation is life threatening, do not leave the laboratory before complete cloth changing and decontamination.
2. PI should be readily informed about any incidents or accidents either with human or environmental impact. If possible, try to limit the damages and/or injuries as well as accidental release of GM material.
3. Reported any facility occurrence in the Access logbook.

Technical failure of equipment or permanent construction problems may be the origin of environmental incidents or may cause accidents. Hence, they must be reported to PI and solved as soon as possible by facility or maintenance staff.

Emergency situations due to weather calamities must be reported to the PI and urgent containment measures taken out to minimize consequences of GM material release.

8. REFERENCES

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