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There is no known risk of pest or pathogen introduction through the import of oil palm clones from Costa Rica

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Introduction

The risk of introducing pathogenic agents (viruses, pests, fungi, etc.) through the import of oil palm seeds is very low. In particular, oil palm seeds produced in Central America can be considered free of such risk, since the only known seed-borne pathogen of this crop, *Fusarium oxysporum* f. sp. *elaeidis*, is absent in the region (Turner 1980; Franqueville & Diabaté 2004; Flood J. 2004). More than 133 million seeds have been exported from Costa Rica since 1986 to more than 30 countries in America, Asia and Africa, without a single interception being reported by the destination countries (Escobar & Chinchilla 2004).

The risk of introducing pests or pathogenic agents through clonal material of the oil palm could be considered virtually non-existent, because tissue culture procedures must be carried out in aseptic conditions, where strict sanitation measures are part of the normal and required procedures. ASD of Costa Rica has more than 20 years of experience cloning the oil palm, and a new, state-of-the art laboratory was recently built where commercial operations started in the year 2000.

The process of cloning the oil palm

In general terms, the process starts with the selection of an exceptional palm (with high oil production potential, for example). This individual palm is called an *ortet*, and tissue excised and processed in the laboratory is called an *explant*. These explants are placed in suitable sterile media to promote the formation of an undifferentiated mass of cells called a callus. This process may take a year, and then this tissue is transferred to another medium to induce embryogenesis (a mass of cells with the potential to differentiate leaves and roots). This process takes several months.

For commercial purposes, enough tissue (embryogenic masses) must be obtained to produce large numbers of plants of any particular clone. This is achieved by seeding the masses of *embryoids* in a proliferating medium. Eventually, the *embryogenic* tissues generate an aerial part (leaves), and then they are induced to form roots (Corley and Tinker 2003). The entire process requires strictly aseptic conditions to reduce contamination to a minimum. The final result is the production of a large number of plantlets called *ramets*, which together form a clone that should duplicate the characteristics of the palm originally selected as an *ortet*.

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Sanitary controls during the cloning procedures

A very low rate of contamination (lower than 0.4 %) is achieved in the laboratory through an integrated air quality-control system, comprised of three aspects: relative humidity (balancing heat energy from illumination and equipment), suspended particles (air filter batteries: cloth, charcoal and HEPAs, and high rates of recycled air), and sanitation (UV lights). Plant tissue is handled in laminar transfer chambers within the clean area which is classified as 10000, which means that no more than 10,000 particles of 0.5 mm/ft3/minute are allowed.

The water for media preparation comes from a deep well and is sequentially filtered to remove suspended particles, hardness, and microelements using a battery of standard pharmaceutical procedures. Sterile media are discharged directly into the clean room by using a two-door autoclave.

ASD's cloning protocol uses immature inflorescences as sources of *explants* (leaves 10 to 16 on the phyllotaxy). These young inflorescences are naturally enclosed within double spathes which keep them virtually sterile, since plants tend to exclude most pathogens from young reproductive organs. Additionally, once in the laboratory, the inflorescences are superficially decontaminated by immersion in sodium hypochlorite, before extracting the tissue that will be eventually used in the regular tissue culture procedure for obtaining clones. In the final stages of the procedure, the *ramets* are transferred from glass vessels into cellophane bags containing a nutritive solution, used to ship the *ramets* overseas.

Sanitary controls during *ortet* selection and collection of *explants*

Selection of *ortets* is done based on data from field experiments, but a particular palm for use is only chosen after close examination to guarantee that the palm is free of any abnormality or known pest or disease that may pose a phytosanitary risk. A strict surveillance system is maintained in all areas dedicated to plant breeding to promptly detect and treat any unusual situation related to pest and diseases.

Ortets grow in disease-free areas, certified as such by an official governmental organization known as the Servicio Fitosanitario del Estado (State Phytosanitary Service). Costa Rica's phytosanitary services operate under the terms of the 'Agreement on the Application of Sanitary and Phytosanitary Measures', whose objective is to guarantee that all export firms meet the highest standards in the phytosanitary monitoring of their product shipments. The certification of export plant product shipments is regulated by Costa Rica Phytosanitary Protection Law 7664 (www.protecnec.go.cr), its associated regulations, the International Plant Protection Convention (IPPC), and the World Trade Organization.

Within these agreements, both the plantation areas and packing plants are subject to periodic visits by official Plant Health Inspectors (PHI). The health inspectors make observations, issue recommendations, and if considered necessary, take samples to be sent to specialized laboratories. Compliance with these

recommendations is mandatory. PHI also carry out inspections and certification of shipments for export at exit points, which is a final requirement for getting the Phytosanitary Export Certificate that accompanies all shipments of seeds and clones from Costa Rica. Packing and transportation are also regulated to meet the standards of both national and international regulations.

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