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PLANT QUARANTINE PROCEDURES FOR EXCHANGE OF ICRISAT SEED

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1. Introduction

As per the Memorandum of Understanding between the Government of India (GOI) and the International Crops Research Institute of Semi-Arid Tropics (ICRISAT), unrestricted movement of seeds of the five mandate crops into and out of India is permitted after observing the National plant quarantine regulations which are governed under the Destructive Insects and Pests Act, 1914. Under this Act, GOI has authorized the National Bureau of Plant Genetic Resources (NBPGR), an agency of the Indian Council of Agricultural Research (ICAR) as the quarantine authority to clear ICRISAT seed. The Government has also recognized ICRISAT Plant Quarantine Unit (PQU) as an Export Certification Laboratory to work under the close supervision and in conjunction with NBPGR. The PQU also liaises with NBPGR for release of imported germplasm addressed to ICRISAT and carries out surveillance for pests and diseases in cooperation with NBPGR, in Post Entry Quarantine Isolation Area (PEQIA) for release of healthy first generation seeds.

2. Quarantine Procedures for Imported Germplasm

All the germplasm material received at NBPGR should be accompanied by a Phytosanitary Certificate (PSC) issued by the National Plant Quarantine Service of the exporting country in the form prescribed by the FAO International Plant Protection Convention of 1951 (Rome Certificate). The Certificate should contain information concerning the health of the seeds, treatments, additional declaration required by the GOI, and a description of the consignment.

2.1. Fumigation

Seed samples received are fumigated prior to subjecting them to inspection as per International seed health testing methods. The seed of sorghum, pigeonpea and chickpea are fumigated under a vacuum at a pressure of 125 mm of mercury with methyl bromide dosage of 32 gm^{-3} for 4 hours. Groundnut and pearl millet seeds are fumigated at normal atmospheric pressure with aluminium phosphide dosage of 3 gm^{-3} for 5 days.

2.2. Radiographic Examination

The seeds of chickpea, groundnut and pigeonpea are examined by X-ray for detection of latent infestation of pests, particularly bruchids. Hidden infestation in sorghum and pearl millet is detected by an Ashman Simon detector.

2.3. Visual Examination

During visual examination, the dry seeds are carefully examined under illuminated floating arm desk magnifiers of 2X magnification for discarding weed seeds, soil clumps, stones, broken/damaged/shrivelled/blemished grain, admixture of plant debris, sclerotia, galls, insects, smut sori, discoloured and mouldy seeds. Apparently healthy looking and clean seeds are selected and those of poor quality are rejected.

2.4. Washing and Sedimentation Test

This test detects fungal spores (i.e., oospores/teliospores, etc) or hyphae adhering to the seed coat. This is used to test fungi that do not grow on seed during incubation, but may be carried externally through seeds as spores. For this test, 50 seeds from each accession are randomly drawn into a separate test tube. Distilled water (10 ml) and 10 to 15 drops of 95% ethyl alcohol or a detergent are added to the test tubes. The tubes are shaken in a mechanical shaker for 10 minutes and the suspension is centrifuged at 3000 rpm for 10 minutes. Discarding the supernatant, the pellet is resuspended in 2 ml of sterile water. The suspension from each test tube is examined under a microscope, utilising bright field.

2.5. Blotter and Agar Media Tests for Detecting Fungi

The blotter test consists of arranging seeds on 3 layers of moist filter paper and incubating them at $22 \pm 2^\circ\text{C}$ under near ultra violet light, with twelve hours alternate cycles of light and darkness for seven days (ISTA, 1976). After incubation the seeds are examined under a stereobinocular microscope at 50X magnification. Petri dishes with specific media (i.e. Agar plate method) are used for detection of specific fungi like *Ascochyta*, *Fusarium*, etc.

2.6. ELISA and Grow out Test for Viruses and Bacterial Diseases

Earlier, screening for seedborne viruses was accomplished by observation of external systems in a grow out test. The groundnut was grown for 6 weeks in an insect-proof screenhouse, indexed for viruses by using a set of indicator plants. Although this method gave reasonable results, it was time consuming and failed to detect symptomless viruses. For the last four years, groundnut germplasm received at the National Quarantine Station are first tested by ELISA for the presence of PMV and PSTV, followed by grow-out test and infectivity assays in an attempt to release the seed consignment expeditiously.

For conducting ELISA test only 10 seeds of each germplasm line are used. A portion of cotyledon approximately 0.2 g of the seed (opposite to embryo) is removed from each of the 10 seeds, mixed and triturated in an antigen buffer and processed by Direct Antigen Coating Enzyme Linked Immunosorbent Assay (DAC - ELISA). In case of positive reaction, individual seeds from each lot are numbered and tested by ELISA. The seeds that give a positive reaction, either to PMV or PSTV, are eliminated from grow-out tests. Seeds that give negative a reaction in ELISA are planted in sterilized soil in a screenhouse and seedlings are observed for 6 weeks. During observation, any plant showing virus disease symptoms is detained and only healthy looking plants are released for growing in the PEQIA. Seeds harvested from healthy looking plants in the PEQIA are released

to scientists. By following such a rigorous procedure, it is possible to exclude seedborne viruses of quarantine importance.

Intermediate /third country quarantine

For cytogenetic studies, wild *Arachis* sp. from the Americas, are first grown in U.K., a non-groundnut growing country, and cuttings from virus-free healthy plants are only imported. After quarantine inspection at the port of entry, they are again grown in an insect-proof screenhouse at ICRISAT for 6 weeks and healthy looking plants are transplanted in PEQIA for seed collection.

Additional requirements for Import of ICRISAT Mandate Crops

As per the national plant quarantine regulations, additional declarations are required to be mentioned in the PSC to safeguard against specific pests and diseases of high risk to the crops of the country. The requirements are as follows:

- **Sorghum.** Certification that the seed samples were collected from fields regularly inspected during active growing season and found free of infection of bacterial leaf stripe (*Pseudomonas andropogoni*) and bacterial leaf streak (*Xanthomonas holcicola*).
- **Pearl millet.** Certification that the parent crop was regularly inspected during its active growth period and was found free of downy mildew (*Sclerospora graminicola*).
- **Chickpea.** Certification that the seed samples were collected from mother plants free of *Ascochyta rabiei*, in addition to viral diseases such as stunt and mosaic.
- **Pigeonpea.** Nil.
- **Groundnut.**
 - Certification that seeds were produced in areas where rust disease (*Puccinia arachidis*) and scab (*Sphaceloma arachidis*) do not occur.
 - Certification that the parent crop was regularly inspected during its active growth period and was found free of peanut stunt, peanut stripe, and marginal and ring spot viruses.
 - Certification that the seeds were treated with an appropriate fungicide at the stated dosage before dispatch.

Seed Treatment

Seeds are treated with pesticides prior to planting. However, seeds intended for specific studies such as disease resistance or response to different strains of *Rhizobium* or chemical analysis are exempted from treatment.

Post Entry Quarantine

All the seed material, after its release by the National Quarantine Authorities is further required to be grown in the PEQIA for first generation under strict

Table 1. Exchange of seed samples and plant materials (1973-1989).

Crop	Export (1974-89)	Import (1973-89)
Sorghum	368 980	77 164
Pearl millet	131 326	25 140
Chickpea	184 548	28 861
Pigeonpea	48 162	8 716
Groundnut	59 359	14 870
Minor millets	12 047	6 174
Total	804 420 (to 147 countries)	*160 925 (from 95 countries)

*This excludes 5000 groundnut cuttings.

5. Future Perspectives and Strategies.

1. For an effective control of the introduction of new diseases, there is an urgent need to have up-to-date information on pests, diseases and weeds of plant quarantine importance, and on outbreaks at the regional and global level.
2. Strengthening of Plant Quarantine Unit by providing tissue culture facilities and sophisticated equipment for quick detection of pests and diseases (including nematodes and weeds) of plant quarantine importance.
3. Facilities for performing immunosorbent assay technique and maintenance of antisera for different viruses and bacterial pathogens.
4. Adoption of new disease indexing techniques.
5. Updating the quarantine treatment schedules, procedures under different environmental conditions.