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INTRODUCTION OF GERMPLASM AND PLANT QUARANTINE PROCEDURES

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ICRISAT's Plant Quarantine System for Germplasm Exchange

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ABSTRACT

The plant quarantine procedures and techniques adopted by ICRISAT for the exchange of seeds of sorghum, pearl millet, pigeonpea, chickpea, and groundnut are described. For the imported germplasm, the procedures include an intermediate quarantine for groundnut cuttings, fumigation, visual and microscopic examination, seed treatment, testing groundnut seeds by enzyme-linked immunosorbent assay (ELISA), and finally growing the seeds in postentry quarantine isolation area (PEQIA) before release to scientists. Similarly, to export the germplasm, regular inspection of crop by scientists during the growing season, collection of healthy seeds followed by fumigation, visual examination and conducting the ELISA test for groundnut are the essential prerequisites before the germplasm is presented to national plant quarantine authorities for issuance of the phytosanitary certificate. During the last 14 years, a total of 152,678 samples of seed and vegetative material of ICRISAT mandate crops were imported from 6 countries, and 701,547 samples exported to 143 countries through the national plant quarantine authorities.

To improve the efficiency of the plant quarantine system in future, the strategies proposed to be explored include (a) use of serological techniques for detecting seedborne viruses, (b) adoption of new disease indexing techniques, and (c) updating information on treatment schedules, procedures, and compilation of information on pests, diseases, and weeds of plant quarantine importance with relevance to ICRISAT's mandate crops.

INTRODUCTION

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, scientific, research and training institute receiving support from donors through the Consultative Group on International Agricultural Research (CGIAR). The Institute was established by CGIAR in 1972 at Patancheru, near Hyderabad, Andhra Pradesh, India. Its mandate includes the collection, evaluation, maintenance, utilization, and distribution of sorghum, pearl millet, pigeonpea, chickpea and groundnut germplasm.

As per the memorandum of understanding between the Government of India (GOI) and ICRISAT, unrestricted movement of seeds of five mandate crops into and out of India is permitted after observing the national plant quarantine regulations. Further, GOI recognized ICRISAT Plant Quaran

tine Unit as an Export Certification Laboratory to work under its close supervision. It authorized the Director of Central Plant Protection Training Institute (CPPTI) located at Rajendranagar, Hyderabad, to act as the Quarantine Authority to clear ICRISAT's seed material of its mandate crops, and the Director, National Bureau of Plant Genetic Resources (NBPGR), New Delhi, for crops that do not form part of ICRISAT's mandate (such as minor millets). In 1975, GOI constituted a committee to review and revise the quarantine arrangements for ICRISAT germplasm to facilitate smooth clearance of germplasm as well as to check introduction of exotic pests/diseases of quarantine importance. In July 1986, GOI authorized the Regional Plant Quarantine Station of NBPGR at Rajendranagar, Hyderabad, as the sole plant quarantine authority, to clear ICRISAT's mandate crops as well. It also instructed the Indian Customs authorities to ensure unrestricted movement of those seeds from the port of entry to NBPGR.

NATIONAL QUARANTINE REGULATIONS

In India, at present, two categories of legislative measures are in operation to control pests, diseases and weeds. They are (a) legislative measures through The Destructive Insects and Pests Act, 1911 (II of 1914), and (b) legislative measures through State Agricultural Pests and Diseases Act.

Under the first category of regulatory measures, GOI took legislative steps as far back as 1914 to prevent entry of pests and diseases into India. The Act was passed by the Governor General of India in Council on 3 Feb 1914 wherein rules governing the import and movement of plants and plant materials, insects, and fungi are framed (3). The second category of the regulatory control deals with the necessary legislation for the control of potentially dangerous and destructive pests, diseases, and obnoxious weeds in India.

The present paper highlights the regulations and quarantine procedures that are followed by ICRISAT for safe exchange of germplasm and the reliability of the present system in minimizing the risk of the introduction of exotic pests/pathogens and weeds.

PLANT QUARANTINE PROCEDURES FOR IMPORTED GERMPLASM

All the germplasm material meant for ICRISAT received at NBPGR from other countries, are accompanied by a phytosanitary certificate in the form prescribed by the FAO/International Plant Protection Convention 1951 (also called the 'Rome Certificate'). The seeds of sorghum, pigeonpea, and chickpea are fumigated under vacuum at a pressure of 125mm of mercury, with a methyl bromide dosage of $32gm^{-3}$ for 4hr; millet and groundnut are fumigated at normal atmospheric pressure with aluminium phosphide dosage of $3gm^{-3}$ for five days (11).

After fumigation the seeds are subjected to the gravity method to detect exotic nematodes, and visually examined to discard weed seeds, soil clumps, stones, broken/damaged seed, and other impurities. In case of sorghum and pearl millet, the hidden infestation by insects is detected by Ashman Seed Infestation Detector and for bruchid infestation in pigeonpea and chickpea the seeds are subjected to radiography.

DETECTION OF FUNGAL AND BACTERIAL PATHOGEN

To detect bacteria and fungi on the surface of the seeds, the following procedures and techniques are adopted:

a. use of a magnifying glass to separate moldy seeds, smut sori and sclerotia;
b. washing/sedimentation methods to isolate fungal spores;
c. blotter test, which consists of placing seeds on moist filter paper and keeping those in an incubation chamber for seven days at 25 °C with alternate light and dark cycle, to detect seedborne fungi, and
d. the use of petri dishes with agar media for detection of specific fungi like *Ascochyta* sp.

DETECTION OF GROUNDNUT VIRUSES

The groundnut seeds are grown for six weeks in an insect-proof screen house and kept under observation for expression of virus disease (peanut mottle, peanut stunt, marginal chlorosis, peanut stripe, and ring spot) symptoms. Plants showing virus-like symptoms are detained and only seedlings looking healthy are released for growing in postentry quarantine isolation area (PEQIA) at ICRISAT Center. For the last two years, groundnut germplasm lines imported into India are also being tested by enzyme-linked immunosorbent assay (ELISA) technique on dry seed, followed by grow-out and infectivity tests. This procedure is found to be quite satisfactory in tackling peanut mottle virus in this crop (12).

ADDITIONAL REQUIREMENTS FOR IMPORT IN RESPECT OF ICRISAT CROPS

As per the national plant quarantine regulations, specific additional declarations are required to be mentioned in the phytosanitary certificate as a safeguard against specific pests and diseases whose introduction can be considered as high risk to the crops in India. The requirements are as follows:

Sorghum. Seed samples should be certified as collected from fields that were regularly inspected during active-growing season and were found to be free from infection of bacterial leaf stripe *(Pseudomonas andropogonis)* and bacterial leaf streak *(Xanthomonas campestris* pv. *holcicola)*. Further, the seed must be free from southern leaf blight *(Dreschlera maydis)*.

Pearl millet. Seeds should be certified as collected from crops free from infection of downy mildew *(Sclerospora graminicola)*

Table 1
Seed treatment schedule for ICRISAT mandate crops

Crop		Treatment	Remarks (if any)	References
Sorghum	i.	Thiram or captan @ 2.5gkg $^{-1}$	Prophylactic measure against seed rot or molds	5
	ii.	Carboxin @ 1.5gkg $^{-1}$	Against smut disease	10
	iii.	Metalaxyl @ 4gkg $^{-1}$	Against downy mildew	1
Chickpea	i.	Mixture of benomyl and thiram (3:2) @ 4.5gkg $^{-1}$	Against wilt disease	2
	ii.	Thiabendazole @ 3gkg $^{-1}$	Against ascochyta blight	8
Pigeonpea		Mixture of benomyl and thiram (3:2) @ 4.5gkg $^{-1}$	Against wilt disease	4
Groundnut		Thiram @ 3gkg $^{-1}$ of seed	Against fungal pathogens	9
Pearl millet	a.	The seeds are soaked in 0.1% mercuric chloride for 10min and then thoroughly washed in running water for 5min. After washing, the seeds are transferred immediately into a water bath set at 55°C for 12min. Immediately after the hot water treatment, the seeds are transferred to water at room temperature for 2min cooling after which they are transferred to incubator at 35°C for 12hr and then at 40°C for an additional 12hr. After cooling the seeds are treated with the fungicide metalaxyl (Ridomil™ 50% wettable powder) at 3gkg $^{-1}$ seed in 1000ml of 1% aqueous methyl cellulose solution. The seeds are soaked in the fungicide suspension for 4-6 hr. The treated seeds are dried under shade/sunlight and can be used for sowing up to a period of four months.	Against downy mildew	6, 7

Chickpea. The seed samples should be certified as collected from plants free from blight *(Ascochyta rabiae)* and virus diseases.

Pigeonpea. There are no specific requirements.

Groundnut. a. Seeds should be certified to be from a crop that was inspected regularly during active growth and found free from symptoms of peanut mottle, peanut stunt, marginal chlorosis, and peanut stripe viruses. b. The mother plants were free from two diseases, i.e. rust *(Puccinia arachidis)* and scab *(Sphaceloma arachidis)*.

The consignment of cuttings of wild *Arachis* species originating from North and South America shall be subjected to intermediate quarantine in non-groundnut growing countries and only healthy seedlings or cuttings shall be imported. At present such cuttings destined for ICRISAT are grown for one generation in greenhouses at the University of Reading, UK. The cuttings from healthy plants upon arrival into India are examined at the Plant Quarantine Fumigation Station, Palam Airport, New Delhi, and are grown in the nethouse at ICRISAT Center under the supervision of NBPGR. The plants free from viruses are then transplanted in PEQIA for seed collection.

SEED TREATMENT

Seed should be treated with pesticides prior to planting. The treatment schedule followed for the seeds of sorghum, pearl millet, chickpea, pigeonpea, and groundnut is given in Table 1.

POSTENTRY QUARANTINE

The seed material released by National Plant Quarantine authorities are grown in PEQIA at ICRISAT for one generation under strict vigilance. The PEQIA is about 50ha located in the South East corner of ICRISAT Center and about 6ha of it is under cropping. This area is about 200m from the nearest crop fields. This area is also surrounded by trees acting as wind barriers. The staff of NBPGR and ICRISAT Plant Quarantine Unit inspect the crops twice a week from sowing to harvest. The crops receive adequate plant protection measures throughout the growing period, and any plants showing symptoms of exotic diseases are immediately rogued and incinerated. Seeds harvested from healthy plants are released to ICRISAT scientists.

Between 1973 and 1987, 74,496 seed samples of sorghum, 21,845 of pearl millet, 28,183 of chickpea, 8,389 of pigeonpea, 13,998 of groundnut, 5,767 of minor millet, and 5,000 groundnut cuttings, totalling 157,678 samples were imported by ICRISAT from 94 countries.

QUARANTINE PROCEDURES FOR EXPORT OF GERMPLASM

The seed export from ICRISAT started in 1974. We strictly adhere to the requirements laid down by the FAO/Plant Protection Convention (1951) for the issuance of phytosanitary certificate for exporting seeds. The GOI

proved the establishment of an export certification laboratory at ICRISAT in 1978 for expeditious movement of germplasm to other countries. ICRISAT provided the laboratory facilities and necessary staff to carry out the initial operations to prepare the seed for export.

ICRISAT's Plant Quarantine Unit is equipped with fumigation chambers, radiographic equipment, an incubation room with near-ultra-violet light, temperature and humidity controls according to DGISP (Danish Government Institute for Seed Pathology for Developing Countries, Copenhagen, Denmark) standards, entomology and plant pathology laboratories, and rooms for receiving, cleaning, inspection, seed treatment, and packing. The laboratory is headed by the Chief Plant Quarantine Officer who is assisted by well qualified technicians in entomology and plant pathology disciplines, and one fumigation assistant besides other support staff.

FUMIGATION

As soon as any seed consignment is received at the Plant Quarantine Unit, it is subjected to vacuuln fumigation with a methyl bromide dosage of $32gm^{-3}$ for 4hr. Pearl millet and groundnut are fumigated under normal atmospheric pressure with aluminium phosphide at a dosage of $3gm^{-3}$ for five days (11).

The seeds are then examined, tested, and treated in the similar pattern as in the case of imported seed before they are presented to the national plant quarantine authority for issuance of a phytosanitary certificate. All seed materials for export are also treated with Aldrin @ $3gkg^{-1}$. Seeds intended for disease-resistance studies or for response to different strains of *Rhizobium* or chemical analysis are exported without treatment.

The seed packages are dispatched by airfreight or airmail, depending upon the weight of the consignment and destination.

NUMBER OF SEED SAMPLES EXPORTED

From 1974 to 1987, 332,505 seed samples of sorghum, 122,025 of pearl millet, 162,973 of chickpea, 37,379 of pigeonpea, 41,500 of groundnut, and 8,165 of minor millet, total numbering 704,547 were exported from ICRISAT to 143 countries.

During these years there has been no report of the introduction of any pest or disease through exchange of ICRISAT germplasm either in India or abroad and the procedures followed by ICRISAT prove that the quarantine system is functioning satisfactorily.

PROPOSED FUTURE STRATEGIES

To improve the efficiency of the plant quarantine system, in future, the strategies proposed to be explored include (a) use of immunosorbent assay technique and different antisera for detecting viruses and bacterial pathogens, (b) adoption of new disease indexing techniques, and (c) updating the treatment schedules, procedures, techniques, and compilation of information on pests, diseases, and weeds of plant quarantine, their distribution and outbreaks at regional and global basis.

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