Quarantine of Plant Genetic Resources

Plant quarantine is a government endeavour, enforced through legislative instruments, to prevent the introduction and/or spread of quarantine pests or o ensure their official control, when any planting material, plant products, soil or living organisms are introduced into a new geographical area. Plant quarantine services facilitate safe introduction of germplasm samples from other countries. Almost all countries regulate the importation of plants/plant products, including germplasm, because of the pest risk posed by such imports into its ecosystems, especially agricultural ecosystems. International and national regulatory/legislative measures are adopted to prevent introduction of exotic pests. Quarantine measures adopted in India for introduction of plant germplasm are detailed hereunder.

LEGISLATIVE FRAMEWORK

- India is signatory to the International Plant Protection Convention (IPPC, 1952) of the Food and Agricultural Organization (FAO) since 1952, which requires each country to establish a National Plant Protection Organization (NPPO) to discharge quarantine functions.
- In India, the Directorate of Plant Protection Quarantine and Storage (DPPQS), Faridabad, under the Ministry of Agriculture & Farmers Welfare, is the NPPO for implementation of plant quarantine regulations as per the IPPC, through the Destructive Insects and Pests (DIP) Act, 1914, amended from time to time. The quarantine of bulk material imported for planting or for consumption is undertaken by the DPPQS, Faridabad and the network of quarantine stations under it located all over the country.
- Under the regime of liberalized trade in agriculture under the World Trade Organization (WTO), to which India is also signatory, the Plant Quarantine (Regulation of Import into India) Order, 2003 (henceforth referred to as the PQ Order), came into force on January 1, 2004 except sub-clause (22) of clause 3 which entered into force on April 1, 2004.
- Any germplasm imported into India, must be accompanied with an Import Permit (IP) and a Phytosanitary Certificate (PC). As per Section 6 (1) and (2) of the PQ Order 2003, only ICAR-NBPGR is authorized to issue IP for import of germplasm,transgenics or genetically modified organisms (GMOs) or living modified organisms (LMOs) (true seeds as well as vegetative propagules) meant for research or experimentation. Further, as per Section 6(3) of the PQ Order 2003, imported consignments of plant germplasm/ transgenics/ GMOs/ LMOs meant for research purposes, are not to be opened at the point of entry, but are required to be forwarded to the Director, ICAR-NBPGR, New Delhi, for its quarantine.
- Quarantine examination is carried out for all germplasm at ICAR-NBPGR, New Delhi, except germplasm indented from southern states of India (Andhra Pradesh, Goa, Karnataka, Kerala and Tamil Nadu), which are inspected at ICAR-NBPGR Regional Station, Hyderabad.
- For importing germplasm including transgenics/GMOs/ LMOs not listed under Schedules IV, V, VI, VII of the PQ Order 2003, a pest risk analysis (PRA) needs to be carried out prior to issue of IP.

- Export inspection and phytosanitary certification of plants and plant products is carried out in accordance with Article IV of IPPC to meet the legal obligations of the member countries.PCs are issued in the model formats set out under Article V of the IPPC in consistence with prevailing quarantine regulations of the importing country.
- The Head, Division of Plant Quarantine, ICAR-NBPGR, New Delhi and Officer-in-Charge, ICAR-NBPGR Regional Station, Hyderabad, are the designated officials from ICAR-NBPGR authorized to inspect, fumigate or disinfect and to grant PC for plant germplasm for export to foreign countries (as per notification No. 8-97/91-PP.I, dated 26.11.93 of the Ministry of Agriculture, GOI) (http://plantquarantineindia.nic.in/PQISPub/docfiles/notify10.htm).

QUARANTINE PROCEDURE FOR IMPORT OF GERMPLASM INCLUDING TRANSGENICs/ GMOs/ LMOs)

Receipt of Import Consignment and Inspection of Germplasm

- All import consignments received from the airport are only be opened in the designated plant quarantine laboratory at ICAR-NBPGR, New Delhi.
- Samples are counted and verified for the accompanying documents such as IP, PC etc.
- The entire sample is subjected to visual and microscopic examination by a team of experts in fungal pathology, bacteriology, virology, entomology, nematology and weed science, in minimum possible time. Quarantine examination is carried out using minimum possible seeds, so that sufficient quantity of seeds are available for release to the indentor.
- For detailed quarantine examination, specialized tests for detection of various pests are used.
- Once a pathogen, insect, nematode or weed is detected, appropriate disinfestation/disinfection treatments such as physical (mechanical elimination) chemical (fumigation) or thermal treatment is given before release of the material.
- Crop species known to carry seed-borne pests (especially viruses) are grown in a post- entry quarantine (PEQ) facility, such as closed greenhouse/polyhouse or an isolated field, that are duly certified by the Inspection Authorities (IAs) notified in Schedule-XI of the PQ order 2003. All plants grown in PEQ facility are kept under surveillance from the time of sowing until harvest, especially to detect and prevent the introduction of exotic pests. Only seeds harvested from healthy plants are released from imported consignments to the indentor.
- Material found to be chemically-treated with pesticides are also be grown in the PEQ facility for expression of disease symptoms. Only harvest from disease-free plants is released to the indentor.
- Quarantine processing of transgenics/ GMOs/ LMOs is be done in National Containment/ Quarantine Facility at ICAR-NBPGR, New Delhi. Besides regular quarantine examination of pests, the germplasm is tested for absence of terminator gene (genetic use restriction technology) using molecular tools.
- For transgenics/ GMOs/ LMOs requiring PEQ, material is released on an undertaking that material is grown in isolation as per DBT guidelines. The PEQ inspection of 30- 45-days-old plants is carried out at indenters‟ site as well as by laboratory testing, by quarantine scientist(s) of ICAR-NBPGR.

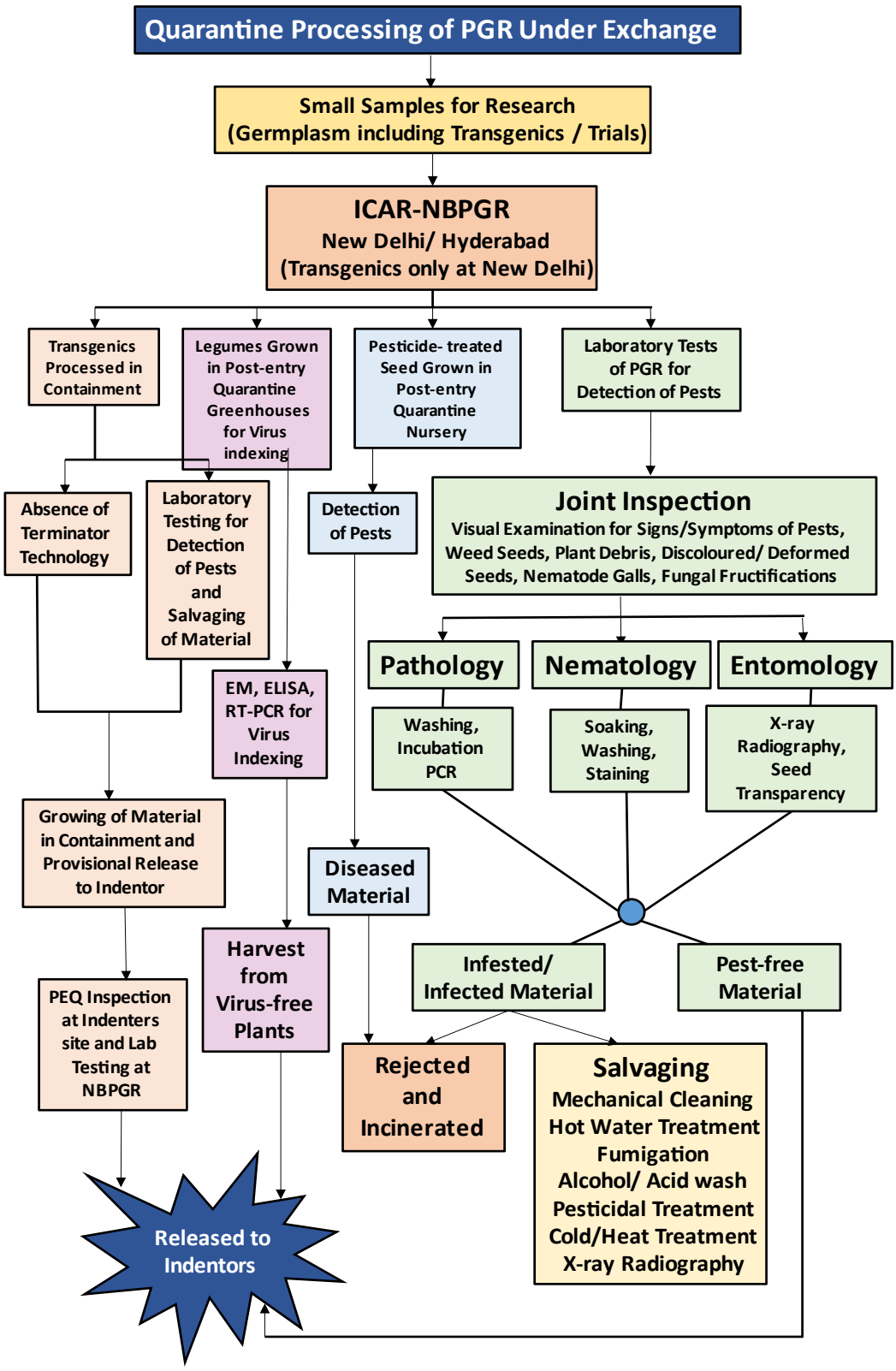

Fig. 1 Schematic presentation of Quarantine Processing

Detection of Fungal, Bacterial and Viral Pathogens

- Blotter test of seeds is used for detection of any fungal pathogen capable of producing mycelial growth, fruiting structures, symptoms on the seedlings and bacterial growth/ symptoms under incubation. Incubated Petri plates are examined under the stereo-binocular microscope on the eighth day.
- Examination of suspension after seed washing are used for detecting surface-borne fungi. The seeds are shaken in water and the resultant suspension is examined for spores of smuts, bunts, downy mildew, powdery mildew and some of the fungi like *Protomyces macrospores* under the stereo-binocular and compound microscope.
- Agar plate method is used for seeds suspected to carry pathogenic fungi and bacteria, wherein seeds are plated on agar plates for detection of fungi/ bacteria and identification is carried out based on types of growth and colony characteristics.
- Seedling symptom test is undertaken for fungi/ bacteria which are capable of infecting seeds, resulting in either rotting or necrosis of seeds or causing symptoms on seedlings.
- Embryo count method are used for detecting embryo-borne fungi capable of causing disease in the plants in the next generation such as loose smut of wheat, downy mildew of pearl millet, etc.
- Molecular methods such as polymerase chain reaction (PCR) is used to detect both fungi and bacteria using species-specific primers.
- Seeds with discoloured or shriveled symptoms could be infected with bacteria. Water-soaked lesions on cotyledons/leaves or streaks on rice leaves or "V" shaped lesions on cotyledons/ leaf in brassicas etc. are initial symptoms of bacterial infection. The bacterial association is confirmed by bacterial ooze test. The associated pathogenic bacteria is isolated on agar medium and identified using morphological, cultural, biochemical, serological and/ or molecular methods such as enzyme-linked immune-sorbent assay (ELISA),PCR etc. Hyper-sensitivity test on tobacco leaves confirms the presence of bacteria. Plant pathogenic bacteria belonging to genera *Erwinia, Pseudomonas, Xanthomonas, Xylella* and *Xylophyllus* are gram-negative while *Clavibacter, Curtobacterium* and *Rhodococcus* are gram-positive. Gram reaction can be confirmed using stains or by KOH test.
- Seeds known or suspected to carry seed-transmitted viruses are grown in insect-proof PEQ facility. Observation of leaf samples showing viral symptoms are carried out under the transmission electron microscope which reveals the size and shape of the virus particles, giving indication about the group to which the virus belongs. The samples are further tested by ELISA and reverse transcription PCR (RT-PCR), if required. Seedlings showing viral symptoms are uprooted and burnt. Produce from only healthy plants are released to the indentors.

Detection of Insects and Mites

- X-ray radiography, a non-destructive technique, is used to detect seeds infested with phytophagous chalcidoids, bruchids and certain other insect groups that do not exhibit any external symptoms on seed surface. A list of 340 plant genera known to carry hidden infestation are compulsorily subjected to X-ray radiography. Upon exposure to X-rays,the seeds clearly show insect infestation which are hand-picked and removed from the healthy

seeds which are then released to the indenter.

- Transparency method is used for detecting infestation in small seeds. If infestation is detected, the entire sample is subjected to treatment for disinfestation.

Detection of Plant Parasitic Nematodes

- Seeds known to/suspected to carry seed-borne nematodes are soaked in water for 24 h. Softened seeds are teased/crushed under a microscope to observe the nematodes, if present.
- Plant material/soil is soaked overnight and sieved through nematological sieves. These are recovered and examined under the compound microscope for identification of nematodes.
- Staining technique is used for quick detection of nematodes in vegetative propagules.The nematodes, if present, retain the red stain more deeply than the plant tissue and can easily be detected under a stereo microscope. Examination of accompanying soil is done to detect the presence of viable nematodes, especially ectoparasites and cysts of cyst forming nematodes.

Disinfection/ Disinfestation of Germplasm

(i) **Mechanical cleaning**: Any soil clods, plant debris, weed seeds, ergot sclerotia, smut/ bunt balls, discoloured, deformed, malformed seeds are mechanically cleaned by hand picking. The vegetative propagules are cleaned by excising the infected portion.

(ii) **Hot water treatment** (HWT): Various temperature and durations is used for eliminating pathogens like fungi, bacteria and nematodes. The treatment is given in hot water treatment tank fitted with heaters of different capacities, stirrer, thermostat and contact thermometer for controlling the water temperature. In some cases, pre-soaking gives better results.

(iii) **X-ray radiography**: It is a unique technique used for both detection and salvaging and is used to separate insect infested seeds (which do not have any external symptoms) from healthy ones. Upon exposure to X-rays, the infested seeds can be easily distinguished and are hand-picked from the seed geometry. In case of real-time X-ray machine, the process is much faster and salvaging can be done immediately after the image of infested sample appears on the monitor screen.

(iv) **Fumigation**: It is one of the most effective methods used in quarantine for eliminating insects and mites. Atmospheric fumigation is done at normal air pressure in an air tight container using approved fumigants.

(v) **Pesticidal treatment**: It is the most practical method in quarantine for effective control of surface feeding insects, mites, nematodes, etc. Pesticidal dip/spray for vegetative propagules and pesticidal dressings for seeds of suitable systemic and contact pesticides are used at various concentrations and time duration.

(vi) **Spirit wash**: It is used for eliminating the seed-borne rust spores of *Puccinia carthami* and *P. helianthi*, the safflower and sunflower rusts, respectively.

(vii) **Acid wash**: Concentrated sulphuric acid is used for destroying the spores of sugarbeet rust (*Uromyces beticola*) adhering to seeds.

(viii) **PEQ isolation growing**: Chemically-treated seed material of international trials and material known to carry seed-borne pathogens are grown in isolation in post-entry quarantine

facility for the detection of seed-borne pathogens. Only healthy seeds from the uninfected plants are released to the indentors.

Prophylactic Treatment

- All imported paddy samples are given prophylactic HWT at 52°C for 30 min against seed-borne fungi, nematodes and bacteria.
- All trial material are given mandatory fumigation with suitable fumigant(s).
- Vegetative propagules are given prophylactic pesticidal dip/ spray treatment dependingon the nature of material.
- All the *Capsicum* spp., *Nicotiana* spp. and *Solanum lycopersicon* samples are given 10% trisodium orthophosphate treatment to prevent introduction of *Tobamoviruses*.

Identification and Documentation of Intercepted Pests

- The pests intercepted are preserved as mounted specimens (in case of insects), devitalised samples (in case of weeds), permanent mounts/photographs (in case of pathogens and nematodes) and as leaf samples (at -80°C or lyophilized) and/ or electron micrographs (in case of viruses).
- Records are maintained in the form of soft copies of images of seeds infested with insects/ X- ray plates for future reference.

Release/ Rejection of Import Consignment after Quarantine Inspection

- The consignment after quarantine inspection, treatment (if required) are released for onward supply to indentor.
- Material requiring PEQ, are released after taking suitable undertaking from the indentor.
- Material that cannot be salvaged from infection/infestation are rejected by the Quarantine Officials and such material is disposed in incinerators designed for the purpose.

QUARANTINE PROCEDURES FOR EXPORT OF GERMPLASM

- The exporter requests for phytosanitary certification through an online system accessible through https://pqms.cgg.gov.in/pqms-angular/home, which has a workflow system for issue of PC
- Head, Division of Plant Quarantine, ICAR-NBPGR, New Delhi and Officer-in-Charge, ICAR-NBPGR NBPGR Regional Station, Rajendranagar, Hyderabad are the Designated Officers authorized for issuance of PC for germplasm of PGR (including agro-forestry species). For forestry genetic resources, Head, Division of Forest Pathology, Forest Research Institute, Dehradun, Uttaranchal, is the designated authority for issuance of PC.
- All the samples are provided by the exporter to the appropriate institute, where they are examined by the Plant Quarantine scientists, especially for absence of pests for which the IP from the importing country requires additional declarations, using various techniques described earlier.
- In case the material is infected/infested with the pest(s) of quarantine significance for the importing country (as mentioned in the IP), the sample are rejected for export.

- In case the material is infected/infested with pest(s), but not of quarantine significance for the importing country, the samples are given standard treatments for salvaging the germplasm, and the treatment indicated in the PC.
- After examination and clearance, a PC is generated through the online system and is printed in colour which are signed digitally by the Designated Officer which would accompany the consignment to the importing country.
- The PC certifies phytosanitary compliance of the material exported with all the requirements of additional declarations and specific treatments as indicated on the IP issued by the importing country.

DOS AND DON'TS

For Indentors

- The minimum quantity of untreated seed required for quarantine processing is about 20 seeds. Therefore, indentors should seek IP for sufficient number of seeds during import.
- The indentor should anticipate the import request well in time, keeping in view the sowing time and season for any material requiring PEQ growing.

For Quarantine Personnel

- While dealing with toxic chemicals during treatments, all precautions should be taken such as wearing of masks, gloves, laboratory coats to avoid any direct contact with the chemical(s).
- All toxic chemicals including fumigants should be stored in a cool dry place and expired harmful chemicals should be disposed off as per guidelines of the GOI.
- X-ray radiography should be carried out only by authorized personnel, who are monitored for radiation safety periodically. Use of Thermoluminscent Dosimeter (TLD) badges is must whileusing the X-rays**,** to monitor the radiation doses received by a person. The personal monitoringshould be in compliance to Radiation Protection Rules, 1971 of GOI under Atomic EnergyAct, 1962.
- The quarantine personnel should follow all the safety norms of working in a containment facility and greenhouse.

SELECTED READING

1. Agarwal PC, R Nath, U Dev and A Majumdar (1990) Quarantine salvaging of safflower seed infected with safflower rust (*Puccinia carthami*). *FAO Plant Protection Bulletin* **38**: 141-43.
2. Bhalla S, ML Kapur, B Lal, BR Verma and C Singh (2002) Quarantine risk associated with exchange of plant genera carrying hidden infestation. *Indian Journal of Plant Genetic Resources* **15**: 160-163.
3. Bhalla S, V Celia Chalam, A Lal and RK Khetarpal (2009) *Practical Manual on Plant Quarantine*. National Bureau of Plant Genetic Resources, New Delhi, India, 204+viii p.
4. Bhalla S, V Celia Chalam, V Tyagi, A Lal, PC Agarwal and IS Bisht (2014) *Teaching Manual on Germplasm Exchange and Plant Quarantine*. National Bureau of Plant Genetic Resources,New Delhi, India. 350 p.

5. Bhalla S, VC Chalam, B Singh, K Gupta and SC Dubey (2018) *Biosecuring Plant Genetic Resources in India: Role of Plant Quarantine*, ICAR-National Bureau of Plant Genetic Resources, New Delhi, 216 p +vi (ISBN 978-81-937111-1-8)

6. Chalam VC (2020) Elimination of plant viruses by certification and quarantine for ensuring biosecurity. In: Applied Plant Virology Advances, Detection and Antiviral Strategies. Awasthi LP (eds) Academic Press., USA, pp 749-762

7. Chalam VC and AK Maurya (2018) Role of quarantine in ensuring biosecurity against transboundary plant viruses. *Agricultural Research Journal* 55 (4): 612-626.

8. Chalam VC and RK Khetarpal (2008) A critical appraisal of challenges in exclusion of plant viruses during transboundary movement of seeds. *Indian Journal of Virology* 19 (2): 139-149.

9. Dubey SC, K Gupta, J Akhtar, VC Chalam, MC Singh, Z Khan, SP Singh, P Kumar, BH Gawade, Raj Kiran, T Boopathi and P Kumari (2021) Plant quarantine for biosecurity during transboundary movement of plant genetic resources. *Indian Phytopathology* 74, 495–508. https://doi.org/10.1007/s42360-021-00375-7

10. Gregerson T (1978) Rapid method for distinction of gram negative from gram positive bacteria. *European Journal of Applied Biology and Biotechnology* **5**: 123-127

11. Gupta K, SP Singh, Shashi Bhalla and DS Meena (2021) Interception of Insect Pests using X-Ray Radiography during Quarantine of Plant Genetic Resources. *Indian Journal of Plant Genetic Resources*. 34(1): 43–53 DOI 10.5958/0976-1926.2021.00006.1

12. ISPM-5 (2022) Glossary of phytosanitary terms available at https://assets.ippc.int/static/media/files/publication/en/2022/06/ISPM_05_2022_En_Glossary_2022-06-02_PostCPM-16InkAmdts_Fixed.pdf

13. Khetarpal RK and K Gupta (2008) Plant quarantine in India in the wake of international agreements: A review. Scientific Publishers (India), Jodhpur, *Review of Plant Pathology* Vol 4 pp: 367-391.

14. Khetarpal RK, A Lal, KS Varaprasad, PC Agarwal, S Bhalla, VC Chalam and K Gupta (2006) Quarantine for safe exchange of plant genetic resources. In: AK Singh, K Srinivasan, S Saxena and BS Dhillon (eds) *Hundred Years of Plant Genetic Resources Management in India.* National Bureau of Plant Genetic Resources, New Delhi, pp 108-139.

15. Plant Quarantine (Regulation of Import into India) Order 2003 (and amendments) available at http://agricoop.nic.in/gazette.htm

16. Ram Nath, PC Agarwal, U Dev and AK Lambat (1986) Quarantine treatment of sugar-beet seed infected with rust (*Uromyces betae*). *FAO Plant Protection Bulletin* **34**: 205-207.