**(DRAFT)**

**REGIONAL IMPLEMENTATION GUIDANCE FOR PHYTOSANITARY MEASURES**

**Phytosanitary Procedures for**

**Seed Health Certification**

**CONTENTS**

|  |  |
| --- | --- |
|  | *Page* |
| INTRODUCTION |  |
| SCOPE |  |
| REFFERENCES |  |
| BACKGROUND |  |
| 1. General information
 |  |
| 1.1 Commercial seed (for field planting) production process |  |
| 1.2 Pest risk management related to seed production process |  |
| 1. Practical phytosanitary measures
 |  |
| 2.1 Pest free areas, pest free places of production, pest free production sites and area of low pest prevalence |  |
| 2.2 Pre-harvest management |  |
| 2.3 Seed cleaning and seed treatment |  |
| 2.4 Seed lot sampling |  |
| 2.5 Seed inspection and testing |  |
| 2.6 Phytosanitary Certification |  |
| 1. Responsibilities of NPPO of seed producing country
 |  |
|  Attachment I: Capsicum seed |  |
|  Attachment II: to be developed |  |

**INTRODUCTION**

**SCOPE**

This Regional Implementation Guidance will provide overarching guidance for the APPPC member countries in implementing ISPM 38 for producing seeds for field planting. This includes descriptions of practical phytosanitary measures that could be used to reduce pest risks in each step of the seed export system.

The attachments to this guidance also provide descriptions and information for each seed commodity including examples of pests associated with that seed commodity, example of accepted efficacious seed treatments accepted in trade, accepted and/or validated diagnostic testing protocols and other pest detection methods.

This Guidance only applies to phytosanitary matters of seeds and does not address seed quality.

**REFERENCES**

**ISPM 5.** *Glossary of phytosanitary terms*. Rome, IPPC, FAO.

**ISPM 10.** *Requirements for the establishment of pest free places of production and pest free production sites*
**ISPM 11.** *Pest risk analysis for quarantine pests*. Rome, IPPC, FAO.

**ISPM 12.** *Phytosanitary Certificates*. Rome, IPPC, FAO.

**ISPM 38.** *International movement of seeds*. Rome, IPPC, FAO.

**ISTA** (International Seed Testing Association). 2019. *International Rules for Seed Testing: Rule 2019*. Zurich, Switzerland, ISTA.

**NAPPO** (North American Plant Protection Organization). 2013. *Phytosanitary guidelines for the movement of seed*. (RSPM) 36. Ottawa, NAPPO.

**OECD.** 2017.*OECD seed scheme: rules and regulations*.

**BACKGROUND**

The international seed trade around the world is growing significantly every year. The Asia-Pacific region is one of the key markets for seed industry countries such as China, India, Japan, and Australia. Asia and Pacific Plant Protection Commission (APPPC) member countries such as India, Indonesia, the Philippines, Vietnam, and Thailand are the main global seed producers and exporters. Importation of seeds for field planting may be associated with risks arising from entry, establishment and spread of seed-borne, seed-transmitted, and contaminating pests when infested seeds are introduced to a favorable environment to the pest. Hence, phytosanitary measures may be necessary. Where measures are necessary, they should be harmonized, as much as possible, among the member countries to optimize pest risks resulting from the movement of seed in the region, noting variations in pest status and Appropriate Level of Protection.

ISPM 38: International movement of seeds, which was adopted by the Commission on Phytosanitary Measures (CPM 12) in 2017, provides useful requirements to assist national plant protection organizations (NPPOs) in identifying, assessing and managing the pest risks associated with the international movement of seeds. However, the implementation of ISPM 38 should be further described to enable NPPOs to identify suitable measures for managing pest risk associated with the movement of seed. APPPC member countries can support seed industries to comply with ISPM 38 by developing a regional guidance to implement ISPM 38.

**PHYTOSANITARY PROCEDURES FOR SEED HEALTH CERTIFICATION**

1. **General information**
	1. **Commercial seed production process**

The quality of the seed production depends heavily on the starting material or parental seeds. The parental seeds are the product of the breeding process done by the breeders. At this stage, the new improved variety may contain the disease resistant gene, drought tolerant gene or the nutritional enhancement genes. Once the new improved variety can be commercialized in the market, parental seeds are sowed or multiplied in case the parental seeds are not enough for the seed production process. The parental seeds are sowed intensively to ensure a high genetic purity. Then the hybrid seeds produced from male and female parent seeds are produced by the growers which can be done through the contracting model or it can be done by the seed producing company (APSA and ISF, 2017 ; Vegetable Seed Production Good Practice Guide).

The contracting process is based on a written or verbal contract between the seed company and individual growers. Under the contract, seed companies specify standards for the production and quality of hybrid seeds to be delivered at a fixed time and price. They provide extension services for crop cultivation, harvesting, and processing to the growers through regular field visits by field production technicians. Cost of all inputs and loans can be deducted from the seed payment. The growers cannot produce hybrid seeds without a contract due to the proprietary germplasm. The quality specification of the product and marketing are also controlled by the companies.

 After the harvesting process is done, the seeds are dried to ensure the moisture content is reduced according to the seed specification of each crop. This process is to ensure that the seeds can be stored for a maximum shelf life. Seeds are usually mixed with other plant materials such as sticks and leaves, dirt, stones, and weed seeds that are collected with the harvested seed. The seed is then cleaned (separated from the other material) by techniques based on differences in weight, size, or shape of the seed. There are many differences in the seed used to make separations including:

 - Size and shape (large vs. small and length, width, and thickness). The most popular way to separate particles of different sizes is by scalping (using a screen which allows the desired seed fall through the specific screen sieve, round and rectangular, while removing the larger particles) or sifting (dropping out smaller particles by using a screen sieve in which only the particles smaller than the seed are allowed to pass).

 - Weight (heavy vs. light and differences in specific gravity and surface area). This separation is best done with a box fan, an air column, aspirator or pneumatic air separator. These work by passing a stream of air past the seed allowing the light (often unviable) seed to be blown out of the seed lot. This method will also remove any light chaff that remains within the seed lot.

 - Surface texture (rough, smooth, or pointed). A flat piece of roughed-up cardboard works well for this separation. Round seed will roll to the bottom when placed at a slight angle while flat seed will be “caught” on the roughed cardboard.

 The final process is seed packaging. After seeds are packed, they must be stored in an appropriate condition to maintain high germination and vigor, or they can be distributed directly to the customers in various market destinations in different countries. The seed conditioning and seed packaging processes can be done in another country or by another company. All processes are done under the quality management system of the seed production company to ensure the high quality of seeds are provided to the customers. A scheme for commercial seed production is shown in Table 1.



 Table 1. Commercial seed production process (APSA/ISF,2017)

* 1. **Pest risk management related to seed production process**

Referring to the seed production process in Table 1, each step should be evaluated for every crop and pathogen combination. There is a potential of infection sources which can be found during the production process as shown in Table 2.



Table 2 Potential Infection sources found during seed production process *(APSA and ISF, 2020 : Good Practices for Healthy Vegetable Seed Production)*

Thus, it is required to measure, control and manage those risk along each production process as following ;

1. Parental seeds in seed production field could be a primary source of inoculum and transmitted diseases to other plants. So, the PRA should be conducted for the imported parental seed, the likelihood of the regulated pest to establish and spread, and economic and environmental impacts.
2. Phytosanitary Certificate from the original country of parental seeds production should be required to declare the seed is complied with the import requirement including seed treatment and seed testing.
3. Inspection of consignment is a general practice of import countries to assure the seed is safe. At the port, seed consignment will be inspected by quarantine officer, and send further to the laboratory to check for seed borne pathogen using standard methods for seed health testing, or post entry quarantine if it needs to grow under controlled condition to observe the symptom. Consignment will release after the test result show no any quarantine pathogens.
4. Post entry quarantine and seed treatment may be conducted by NPPO before release imported parental seed to the field for seed production if there is any suspicion on pest risk.
5. Usually, program of seed production conducted by seed company may include the practices related to reduce pest risk in pre-harvesting stage such as hygiene measures (e.g. disinfection of workers’ hands and shoes, farm equipment, machinery and tools), field inspection, field sanitation (e.g. removal of symptomatic plants, removal of weeds), protected environments (e.g. glasshouses, growth chambers) and crop treatment.
6. Field inspection by NPPO or authorized entities should be conducted at least 3 stages of plant development: pre flowering, flowering, and after flowering before harvest. The result of official inspection is an important evidence to declare for seed health certification.
7. Seed treatment is usually provided as postharvest practices for seeds to reduce the risk from seed borne pathogens which can be spread from infected or infested seed or infected plant debris to the whole seed lot during harvesting and seed processing.
8. Quality seed testing is the most recognized measures to guarantee the produced seed is free from regulated pest concern.
9. Since seeds may be stored for many years before being exported or re-exported, official phytosanitary information on the seed lot should be in place to maintain the integrity and trace the identity of the seed, including in the case of re-export the original phytosanitary certificate for export, should be retained by the NPPO of producing country.
10. **Practical phytosanitary measures**

**2.1 Pest free areas, pest free places of production, pest free production sites and area of low pest prevalence**

 It is highly important to select the pest free areas for seed production. The field history should be recorded each time when the seed production activities are conducted at the field. The record of crop used, sowing date, harvested date and seed transmitted diseases found in previous crop should be checked or tracked to ensure that the field is free from seed transmitted pests before starting the production process. Soil from the field should not be transported from one field to another field. This is to present the contamination of healthy crops and fields that may contain pests. *(APSA and ISF, 2020 : Good Practices for Healthy Vegetable Seed Production)*

* 1. **Seed production and Pre-harvest management**
		1. **Used of healthy seed for seed production**

The seed used for seed production should be free from pest and seed transmitted diseases. Its health should be tested before use and, if pest or disease organisms against which there is an effective seed treatment are present, that treatment should be applied, or the seeds should not be used for seed production.

* + 1. **Field selection**

Field of seed production must be free from volunteer plants and should be isolated from neighboring fields. Seed production in glasshouses may be sufficiently free from volunteer plants to avoid contamination of the crop seed by any seed which is difficult to remove from the crop seed, cross-pollination and importantly seed-borne diseases transmitted from volunteer plants.

Seed crops shall be isolated from all sources of pollen contamination and seed-borne diseases (including seed-borne virus infection and wild plants that might serve as a source of disease). For example, Seed borne diseases affected capsicum seeds, the distances must not be less than 250 m. for certified seed.

The history of selected site should be recognized such that there is the least possible risk of any soil-borne diseases being present which could subsequently be transmitted to the new planting for seed production. If any previous crops have made the pest risk, adequate measures must be taken.

In the USA, seed growing areas have been shifted to dry pacific regions for crops such as cabbage, turnip, beans, and peas for obtaining disease-free seed and indirectly controlling diseases as black leg and black rot of cabbage and turnip, etc. Change in sowing or planting dates is helpful to minimize disease occurrence. (Gupta and Kashyap, 2020)

* + 1. **Crop treatment and hygiene measure in the field**

The many strategies, tactics and techniques used in crop treatment with the aims of prevention and therapy.

Prevention strategies applied before infection (i.e., the plant is protected from disease), the second is therapy or curative strategies with any measure applied after the plant is infected. (i.e., the plant is treated for the disease). An example of the first principle is enforcement of quarantines to prevent introduction of a disease agent (pathogen) into a region where it does not occur. Seed treatment to prevent soil borne infection in the field and hygiene practices for both equipment used in the field and worker should be enforced.

 The second strategies such as chemotherapy is the application of chemicals to an infected or diseased plant that stops (i.e., eradicates) the infection. For example, antibiotics have been infused into plants to reduce severity of phytoplasma diseases of palms (lethal yellowing) and pears (pear decline) but in all cases the chemotherapeutant must be reapplied periodically. There also are some “systemic” fungicides such as the sterol biosynthesis inhibiting (SBI) and demethylation inhibiting (DMI) fungicides that diffuse into the plant tissues to some extent and eliminate recently established infections. (Maloy, 2005). However, most of chemotherapy are mainly fungicides which can not be applied with other importants seed borne pathogens including bacteria, virus and viroids. The other alternative measures must be used as combination such as roguing of infected seedling, control of insect vectors, field sanitation, etc.

 The choice of hygienic practice depends on the specific disease that was present in the field during the previous season. Example of hygiene measure that could be applied in the field including:

* Remove infected plants or plant parts as soon as symptoms appear, collect, bag and destroy or pile diseased material away from fields.
* Removing infected fruit and plant debris from the field can reduce the amount of pathogen inoculum that could move into healthy plant parts.
* Cull piles should be placed away from production fields and waterways and, if possible, covered with a plastic tarp to speed up microbial decomposition and minimize pathogen spores from escaping.
* Burn, chop and spread, or deep plow debris at the end of the season.
* Clean tools during use, disinfecting knives, shears and other harvesting tools often. To accomplish this, wash tools with soapy water and dip or wipe in 70 percent ethanol. It is important to refresh sanitizing solutions as specified on the product label.
* Tool sanitation and hand-washing can help minimize plant-to-plant spread of diseases caused by several bacteria and viruses.
* Provide hand-washing stations equipped with clean water and soap.
* During harvest, careful hand-washing is critical to minimize plant pathogen spread. For example, *tobacco mosaic virus* (TMV) can be transmitted to tomatoes and peppers if hand-washing is poor after smoking cigarettes. This tobacco virus is very stable and can be present on dry tobacco in cigarettes.
	+ 1. **Field inspection**

 Field inspection is one measure that importing country prefer to require in import requirement. This measure could be used to monitor for regulated pests that produce visible symptoms on plant and provided a background information on seed-borne and seed-transmitted pathogens on that field. The NPPO of seed producing country should conduct the field inspection during the growing season. The field inspection pattern should ensure that all parts of the field are adequately and proportionately represented. Where visible symptoms are detected during a field inspection, an appropriate number of plant samples, i.e. suspected plant parts, should be taken for laboratory confirmation by visual inspection and testing. Samples of suspected symptoms or infected tissues should be properly kept and correctly labelled to indicate date, time, locations, crop, plant part and name of collector. All parts and rows should be covered and crossed by the inspector on foot. The walking in the field should be done in a schematic pattern so that the maximum area possible can be covered (see Figure 1).



Figure 1. Walking patterns for field inspection (Feistritzer, 1975)

However, If the plant population in the seed field is so thin that the entire population is less than the number required for taking counts in a schematic manner, the entire population should be counted and walked through.

**2.2.5 Seed cleaning and seed treatment**

 - Seed cleaning process

Measure should be applied in this process regardless of method being used to prevent mixing of batches as well as to present cross-contamination of healthy batches with potentially infected seeds and plant parts

 - Seed treatment

Seed treatment refers to the exposure of the seeds to certain physical, chemical or biological agents to reduce pest incidence of the seeds and treat as a pest control (seed protectant or seed enhancement), particularly during seed storage, seed germination or early growth of the plant. Seed treatment technology has been undergoing rapid changes concomitant with the development of new concepts, methods, materials, machines, growing structures, and cultural practices. Seed treatment can never be an independent technology but rather involves a series of procedures from seed harvesting to sowing. Treatment technology in horticultural crop seeds are extremely variable and expensive and the germination behavior is much more complicated mainly because of its extreme diversity of crops, species, and cultivars (Lee,2004). These treatments are used to protect seed from soil borne infection in the field and also eradicate seed borne infection with no effect on seed germination.

1. Physical treatments (e.g., dry heat, steam, hot water or irradiation by ultraviolet light)

Physical treatments consist of heat treatments of seeds, with the most common being hot water, hot air and electron treatments. Thermotherapy inactivates or kills the pathogen, while it leaves the host tissue viable. Among these physical treatments, hot water treatment is a long-known technique that consists in the immersion of plant material in agitated water at a predetermined temperature and time. Spinach seed lot infested with *C.* *variabile, S. botryosum*, and *V. dahliae* could be treated in 50ºC water for 20 min to eradicate C. variabile, reduce the incidence of *S. botryosum* and *V. dahliae*, and largely eradicate *Fusarium, Alternaria*, and other *Cladosporium* spp. with no adverse effect on seed germination.(de Toit,2005). The hot water and dry heat treatment is a useful and effective disinfection method of seeds for controlling the seed borne diseases in vegetables, although the treatment should be carefully performed to maintain the germinability of seeds.

 2) Chemical treatments (pesticide, disinfectants) and biological (extract) treatment: When treating vegetable seeds, it is critical to follow the directions exactly, because germination can be reduced by the treatment and/or the pathogen may not be completely eliminated. The effect of a treatment on germination should be determined on a small lot of seeds prior to treating large amounts of seed. Proper application of chemical is essential as too much chemical can result in phytotoxicity and inadequate coverage can result in poor stand. The chemical used for seed treatment should follow requirement of importing country.

Table 3. Some common vegetable seed treatment products registered for use in the United

 States as of April 2020

|  |  |
| --- | --- |
| **Active ingredient (Product)** | **Help protect against** |
| Bacteriacides |
| Streptomycin sulphate(AS 50 Agricultural Streptomycin) | Halo blight of bean |
| Fungicides |
| Azoxystrobin(Dynasty fungicide) | Seedborne and soilborne fungi that cause decay,damping- off and seedling blight including species of Rhyzoctonia |
| Captan (Captan 4L ST Flowable Seed Treatment Fungicide) | Seedborne and soilborne fungi that cause decay,damping- off and seedling blight |
| Carboxin (Vitavax Flowable Fungicide) | Seed decay,damping- off fungi, including Rhizoctonia solani and seedborne smut of corn |
| Carboxin+thiran(Pro-Gro Dust Seed Protectant) | Onion smut |
| FludioxonilMaxim 4FS Fungicide | Seedborne and soilborne fungi that cause decay,damping- off and seedling blight |
| Iprodione (Nevado 4F) | Alternaria species |
| Mefenoxam(Apron XL LS) | Damping off and seed rots caused by Pythium, systemic downy mildew in garden pea and sweet corn |
| Mefenoxam+difenoconazole(Dividend Fungicide) | Suppression of post emergent die-back complex and damping |
| Metalaxyl(Allegiance FL Seed Treatment Fungicide) | Pythium damping-off, early season Phytophthora and systemic downy mildew in bean. |
| ThiabendazoleMertect 340-F Fungicide | Seedling disease caused by *Fusarium* spp. Verticillium wilt of spinach,Phomopsis seed decay,seedling wilt,damping-off of bean,and ascochyta blight of pea |
| Thiram(42-S Thiram Fungicide) | Seed decay, damping-off,and seedling blight caused by many seedborne and soilborne organisms. |

Sources: Vegetable Seed treatment (2020)

Seed treatment may be used as a single measure or in combination with other measures to ensure pest risks are mitigated to an acceptable level. A combination of seed treatments can also be applied and seed treatment record by seed producers or manufacturers should be available for importing country.

* 1. **Seed lot sampling**

Sampling of seeds is usually done for visual inspection and/or laboratory testing to examine the present or absent of regulated pests according to the requirement of destination country. Seed testing organizations such as the International Seed Testing Association (ISTA) has developed specific rules and procedures for seed sampling. Furthermore, Guidance on the sampling of consignments for inspection is given in ISPM 31. The test result will be applicable to only a representative seed sample or the whole lot, it depends on who withdraws the sample. So, the method of seed sampling is important before sending submitted sample to laboratory for testing.

Where possible, an inspector should carry out inspection by random sampling to ensure that all sample units have an equal probability of being selected from the lot or consignment. In case the samples for quality checks have already been taken from consignments according to ISTA rules, the phytosanitary inspection can be performed on a subsample from the composite sample of quality checks.

* + 1. **General lot sampling**

Seed lot size is generally not a constraint when a distribution of contaminated/infected seed in the lot is relatively homogeneous. If the distribution is heterogeneous, increased sampling intensity is required. Sample size is determined by damage threshold (in a tolerable infection level) for the pathogen and the probability of detection desired, commonly 95 or 99%. The probability of detection at a given damage threshold is greater as sample size increases, and this probability and the appropriate sample size can be determined by statistical methods as indicated in ISPM 31.

Most seed health tests utilize qualitative data based on the presence or absence of the pathogen in the test sample, with the lot being rejected if the pathogen is detected in the sample and accepted if the sample is negative. So, the greatest number of seed tests for each pathogen especially for bacteria and virus are different which are related to level of infection in seed sample, whereas, for fungi, the number of seed tests is usually not less than 400 seeds per sample. (ISTA, current edition).

 **2.3.2. Small lot sampling**

 The hypergeometric distribution is appropriate to describe the probability of finding a pest in a relatively small lot. A lot is considered as small when the sample size is more than 5% of the lot size. In this case, sampling of one unit from the lot affects the probability of finding an infested unit in the next unit selected. Hypergeometric-based sampling is based on sampling without replacement. It is also assumed that the distribution of the pest in the lot is not aggregated and that random sampling is used. This methodology can be extended for other schemes such as stratified sampling. (ISPM 31 appendix 2.; Cochran, 1977)

* 1. **Seed inspection and testing**

The NPPO of seed producing country should inspect or test the sample for the presence of regulated pests and regulated articles before issuing phytosanitary certification. It depends on the requirement of destination country.

* + 1. **Seed inspection**

Visual inspection should be designed to detect live insects, disease symptoms, soil, plant debris, contaminant seeds and foreign materials (e.g. animal feces, feathers). Guidance on inspection is given in ISPM 23 (Guidelines for inspection). Personnel should have technical qualifications and competencies in pest detection, including knowledge of, access to or capability in identification of pests, plants and plant products, which can be demonstrated through training materials and records.

* + 1. **Seed health testing**

Seed health testing is an essential management tool for the control of seed-borne and seed-transmitted pathogens and continues to be an important activity for their regulation and control through phytosanitary certification and quarantine programs in domestic and international seed trade (Morrison,1999). Seed health testing is also critical for ensuring the health of basic seed stocks used for seed production and for plant germ plasm utilized in research and product development. Seed sampling is the first critical activity in a reliable seed health testing program. Seed health tests are almost always performed on a sample of seed taken from the seed lot. It is, therefore, crucial that the sample used for testing be a representative of the seed lot, and this requires standardized sampling procedures. Seed testing methods can be referred to ISTA Seed Health methods and ISHI-Veg under International Seed Federation (ISF)

* 1. **Phytosanitary Certification**

Phytosanitary Certificate for seed consignment will be issued according to the request of seed company and will be complied with import requirement of destination country. A phytosanitary certificate or a phytosanitary certificate for re-export may be endorsed with any required additional declaration to certify that the seed lot is free from regulated pests of concern. In some case, phytosanitary certificate needs to be attached with a laboratory report according to the importing country’s requirement.

Example of import requirement for pepper seed export to Thailand is as follow;

A Phytosanitary Certificate (PC) or a re-export phytosanitary certificate issue by the National Plant Protection Organization from the exporting country is required. The original copy must accompany every consignment to the Kingdom of Thailand and bear one the following additional declaration, or a combination of the two declarations addressing each of the four quarantine pests:

1. “ The consignment of capsicum seeds was produced in (country) where *Clavibactor michiganensis* sp. *michiganensis*, *Tomato brown rugose fruit virus, Columnea latent viroid* and *Potato sphindle tuber viroid* are not known to occur” OR
2. “ The consignment of capsicum seeds was officially tested using appropriate methods and found free from *Clavibactor michiganensis* sp. *michiganensis*, *Tomato brown rugose fruit virus, Columnea latent viroid* and *Potato sphindle tuber viroid*”

Example of import requirement for pepper seed export to Australia is as follow;

The capsicum seed must have been tested and found free of pathogens of biosecurity concern. Consignment accompanied by phytosanitary certificate with additional declaration and laboratory report

1) For *columnea latent viroid*, *pepper chat fruit viroid* and *potato spindle tuber viroid*, the following additional declaration should be used:

“The capsicum seed in the consignment in lot(s)[insert lot numbers], of name [insert name], grown in [insert name of country], was tested for *columnea latent viroid*, *pepper chat fruit viroid* and *potato spindle tuber viroid* and was found to be free of these viroids by using a reverse transcription PCR test for the viroids on a sample of 20,000 seeds (or 20 per cent of small seed lots) drawn from the lot and divided and tested as sub-samples of no more than 400 seeds.”

2) For *tomato brown rugose fruit virus* and *tomato mottle mosaic virus*, the following additional declaration should be used:

“The consignment of [botanical name(s) (Genus species)] comprises [insert seed lot number(s)] seed lot(s); for each seed lot, seeds were tested by PCR using primers and protocols approved by the Australian Department of Agriculture, Water and the Environment at [insert laboratory name(s) and report number(s)] on a sample size of 20,000 seeds (or 20 per cent of small seed lots) as sub-samples of no more than 400 seeds and found free from *tomato brown rugose fruit virus* (ToBRFV) and *tomato mottle mosaic virus* (ToMMV)."

1. **Responsibilities of NPPO** **of seed producing country**

The NPPO of origin country of seed production is responsible for:

1. Registration of seed producers and manufacturers. This may be renewed on an annual basis after an audit at the beginning of the season.
2. Approving the pest management practices used in seed production or the control system for seed processing or re-packing by producers or manufacturers to ensure that the risks of regulated pests have been mitigated.
3. Monitoring or auditing production measures in the field and the control system of producers or manufacturers during their operational seasons to ensure their continued compliance with requirements, pest management practices and the requirements of the importing countries or destination countries. Frequency and timing of the monitoring or auditing should be determined according to the pest risks, phytosanitary import requirements and records of non-conformity.
4. Conducting field inspection, testing of suspected plants and verification of the documentation and pest management practices.
5. Conducting inspections, sampling and testing of consignments of seed for export.
6. Issuing phytosanitary certificates to confirm that all seed consignment being exported comply with the phytosanitary requirements of the country of destination.

**Attachment I**

**Capsicum seed**

This attachment only applies to the seed of *Capsicum* spp. and does not apply to vegetative plant parts of species under this genus. The pests and measures identified are not intended to be exhaustive.

1. **General information**

Capsicum is an economically important genus of the *Solanaceae* family and contains at least 32 species native to tropical America. Five species are widely cultivated in different regions of this continent: *C. annuum* L. is cultivated in Mexico and Northern Central America; *C. chinense* is planted in the West Indies, Northern South America, and the Amazon region; *C. frutescens* L. is mainly grown in the Caribbean and South America; *C. baccatum* L. is propagated in Peru and Bolivia; and *C. pubescens*, more tolerant to low temperatures, is found at high altitudes in the Andes (Basu & De, 2003) In 2019, the global production of Capsicum reached approximately 38 million tons of fresh fruit and China had the highest production worldwide (18,978,027 tons), followed by México (3238245 tons) (FAO, 2019). Capsicum is used in all forms starting from fresh green fruit with ripe fruit as well as its dried and powdered form. Fresh green pungent fruit is generally used in salads, stuffing, and as a flavouring agent in cooked meals. The non-pungent varieties are cooked as vegetables or processed with other food items to enhance flavour (Welbaum, 2015). Though being an important spice crop grown worldwide, many constraints such as plant diseases have decreased production, causing significant reduction in yield and seed production.

**2. List of regulated seed borne and seed transmitted pathogens for Capsicum seed**

**Table 4** List of seed-borne and seed-transmitted plant pathogens which are related to capsicum seed production from PRA of many countries, their detection, and management.

| **Pest** | **Common name** | **Scientific name** | **Detection** | **Disease management** | **References** |
| --- | --- | --- | --- | --- | --- |
| Fungi | Anthracnose | *Colletotrichum acutatum* | Incubation method (blotter method) | A quality systems approach in production of the seeds by crop inspections and careful selection of healthy fruit should reduce the chances of this organism being associated with the seed. Commercial harvesting (avoiding infected fruit), cleaning and sanitization of pepper seed would reduce the potential for this organism being associated with the seed. | Diao,et al.(2017)Chigoziri.and Ekefan ( 2013) |
|  |  | *C.capcisi* | Incubation method | A quality systems approach in production of the seeds by crop inspections and careful selection of healthy fruit should reduce the chances of this organism being associated with the seed. Commercial harvesting (avoiding infected fruit), cleaning and sanitization of pepper seed would reduce the potential for this organism being associated with the seed. | Chigoziri and Ekefan (2013) |
|  | Anthracnose | *C. gloeosporioides* | Incubation method (Blotter method) | A quality systems approach in production of the seeds by crop inspections and careful selection of healthy fruit should reduce the chances of this organism being associated with the seed. Commercial harvesting (avoiding infected fruit), cleaning and sanitization of pepper seed would reduce the potential for this organism being associated with the seed. | Welideniya, et.al. (2019)  |
|  | Anthracnose | *Colletotrichum coccodes* | Incubation (Blotter method) | A quality systems approach in production of the seeds by crop inspections and careful selection of healthy fruit should reduce the chances of this organism being associated with the seed. Commercial harvesting (avoiding infected fruit), cleaning and sanitization of pepper seed would reduce the potential for this organism being associated with the seed. | Welideniya, et.al. (2019) |
| Bacteria | Bacterial canker | *Clavibactor michiganensis* subsp. *Michiganensis* | No references found indicating a seed test exists for Clavibactor michiganensis pv. michiganensis on pepper. | Management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot Ten-minute immersion of seed in 300 mmol l−1 acidified nitrite resulted in 98% being pathogen free. (Kasselaki et al.,2011) | Cristina et al. (2018) |
|  | Bacterial spot | *Xamthomonas euvesicatoria* | DNA/RNA based, Seed wash;ISF(2017) | Management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot. | Potnis et al. (2015)  |
|  |  | *X. camprestris* pv. *vesicatoria* | An ISHI-Veg method is described ISF (2019) | Management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot. | Shama,(2017). ; Marčić, et.al. 2015 |
|  |  | *X. gardneri* | An ISHI-Veg method is described ISF2019 | Management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot. |  |
|  | Virus  | Alfalfa mosaic virus | No references found indicating a seed test exists for AMV on pepper. (ISF 2019) | A quality systems approach in production of the seeds by crop inspections should reduce the chances of this organism being associated with the seed. | Abdalla and Ali (2012) |
|  |  | Cucumber Mosaic Virus | Pathway not prove (ISF 2019) | A quality systems approach in production of the seeds by crop inspections should reduce the chances of this organism being associated with the seed | Ali, and Kobayashi, 2010 |
|  |  | Pepper mild mottle virus  | Available information indicates there is no scientific basis for regulation of PMMsV on pepper seed.(ISF 2019) | A quality systems approach in production of the seeds by crop inspections should reduce the chances of this organism being associated with the seed | Tanzi et.al.,1990Kenyon et.al. (2014)  |
|  |  | Tobacco mosaic virus | An ISHI-Veg method is described (ISF2017)  | Seed is a known pathway for TMV in pepper and the recommended management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot. | Kenyon, et.al. ( 2014)Avgelis (1986)Tosic et.al. (1980) |
|  |  | Tomato mosaic virus | An ISHI-Veg method is described. (ISF2017) | Seed is a known pathway for ToMV in pepper. Management strategy is to evaluate seed productions by field inspection or seed testing of a representative sample of each seed lot. | ISF (2017) |
|  |  | Tomato brown rugose fruit virus | An ISHI-Veg method is described. (ISF2019) | A quality systems approach in production of the seeds by crop inspections should reduce the chances of this organism being associated with the seed. | ISF (2019) |

Detail of detection method of seed-borne and seed-transmitted pathogens in table 4 could be refer to ISF. These detection methods also indicated the number of seed used for testing from the seed samples for each pathogen.

**3. Disease management of seed borne and seed transmitted pathogens of Capsicum**

3.1 Crop treatments for different seed borne and seed transmitted pathogens.

*1) Fungi*

The main strategy is to prevent outbreaks of the disease in the seedbed or field by using healthy seed. Parent seeds must be verified to be free from seed borne dieseases and treated with physical or chemical treatments before planting. Field inspection of plants at different growing stages and do rogueing of diseased plants to remove a source of inoculum. Plants should be well spaced, good watering and avoid extreme humidity. A balanced fertilization with potassium phosphate and magnesium can help reduce the disease, while excess of nitrogen should be avoided. Traditionally, recommended fungicide for control of anthracnose disease is manganese ethylene bis dithiocarbamate (Maneb) (Smith, 2000) and carbendazim, though the use of both fungicides has been found ineffective under severe disease outbreak. The chemical fungicides generally recommended for controlling anthracnose disease are based on copper compounds, dithiocarbamates, benzimidazole and triazole compounds (Waller, 1992). Newer chemicals-liked, strobilurins based fungicides (e.g., azoxystrobin, pyraclostrobin) have also been used for its management. However, only a few reports are available using this class of fungicide controlling capsicum anthracnose under large field trials (Schilder et al., 2001; Lewis and Miller, 2003; Chen et al., 2009).

*2) Bacteria*

The main strategy is to prevent outbreaks of the disease in the seedbed, by using healthy seed, maintaining strict hygienic precautions, and rotating sites used as seedbeds. It is advisable to sterilize the soil and tools used in growing planting material. If infected plants are found in the seedbed, they should be destroyed. If, despite these precautions, outbreaks occur in the field, very little can be done to prevent losses. (ISF 2019)

Most seed-borne bacteria such as Xanthomonas spp. is either endemic or introduced into field production areas via infested seed or contaminated transplant materials (Jones et al., 1986; Sijam et al., 1991). Thus, the primary management strategy should include the use of pathogen-free, certified seed or disease-free transplant materials (Ritchie, 2000). Preventative measures, such as treating seed with hot water, can significantly reduce disease inoculum in the seeds. In addition, field inspection, removal of potential inoculum sources, such as volunteer plants and infected host plants, should be carried out on a timely basis. Field isolation from infected host plants in closed proximity, accompanied by sanitation, physical removal and disposal of diseased crop material, and crop rotation with non-hosts, should be followed for a disease management (Goode and Sasser, 1980; Ritchie, 2000).

Chemical control has been a primary focus in pest management strategies for bacterial spot of tomato and pepper. Copper based bactericides have been used extensively for disease control.

*3) Virus*

The main strategy is to prevent outbreaks of the disease in the seedbed or field by using healthy seed. Field inspection of plants at different growing stages and do rogueing of diseased plants to remove a source of inoculum. It is important to provide well balanced nutrients and to avoid over-fertilization with nitrogen. In heavily infested areas, early ripening cultivars should be grown. The strategy of control relates mainly to the type of transmission. Plant hygiene is very important for the control of the stable, mechanically transmitted such as TMV.

Soil should as far as possible be free from debris. Contact between the plants and machinery used in cultural operations should be avoided. Disinfect tools, propagating material and equipment with sodium hypochlorite (1 per cent solution of a 12 per cent concentrate of pool chlorine). For the insect-transmitted viruses, the appropriate isolation distance should be kept or insect proof net house, mainly to reduce risk from transmission such as CMV. The control of the vectors with insecticides is used to restrict the spread of viruses and reduce direct damage. Also, remove volunteer or wild tomato plants that could act as reservoirs for the virus. Other potential reservoir plants include fat hen (*Chenopodium murale*), quinoa, (*Chenopodium quinoa*), Petunia hybrids (garden plants) (ISF2019). Integrated control measures of some seed borne viruses are shown in table 5.

|  |
| --- |
| **Table 5.** Strategies useful for managing some viral diseases of pepper  |
| Management Strategy | Pepper mild mottle virus | Tobacco mosaic virus | Tomato brown rugose fruit virus |
| Host resistance | ✔ | ✔ | ✔ |
| Plant disease free or treated seed | ✔ | ✔ | ✔ |
| Crop rotation | ✔ | ✔ |  |
| Avoid handling seedling | ✔ | ✔ | ✔ |
| Greenhouse sanitation | ✔ | ✔ | ✔ |
| Produce seedling in netted house |  |  |  |
| Remove infected seedling | ✔ | ✔ | ✔ |
| Use reflective mulch | ✔ | ✔ | ✔ |
| Eliminate volunteer and weed hosts | ✔ | ✔ | ✔ |
| Avoid handling/injuring in the field | ✔ | ✔ | ✔ |
| Mineral oil application |  |  |  |
| Destroy crop promptly after harvest | ✔ | ✔ | ✔ |

**3.2 Seed treatment for seed borne and seed transmitted pathogens**

Bacterial, fungal and viral plant pathogens can be introduced into a crop on or within seeds. Generally, the earlier the pathogen comes into contact with the crop, the greater the potential for a disease problem to develop. Seed treatments are an effective means of preventing seed borne bacterial diseases and damping-off diseases.

3.2.1 Hot water treatment

By soaking seed in hot water, seed-borne fungi and bacteria can be reduced, if not eradicated, from the seed coat. Hot water soaking will not kill pathogens associated with the embryo nor will it remove seed-borne plant viruses from the seed surface. Hot water treatments will aid in general control of seed-borne pathogens pepper. It will aid in controlling fungi such as Alternaria, Fusarium, and Verticillium.

3.2.2 Chlorine bleach treatment

Treating seeds with a solution of chlorine bleach can effectively remove bacterial pathogens and some viruses (i.e. *Tobacco Mosaic Virus*) that are borne on the surface of seeds by preparing a 946 ml of fresh Clorox® bleach to 4.7 L of potable water. Add seed to the disinfectant solution (100 gm of seed per 834 ml of disinfectant solution and agitate for 1 minute. Rinse the seed in cold water bath for 5 minutes to remove residual disinfectant and dry on a clean paper towel (Lewis,2013).

3.2.3 Hydrochloric acid treatment

Tobacco mosaic virus can be transmitted from one generation to the next on the seed of pimiento peppers. The virus was associated with the seed coats but rarely with the endosperm and embryo. Immersing seeds in 9% hydrochloric acid for 30 minutes has also resulted in elimination of TMV from seed (Demski 1981).

3.2.4 Trisodium phosphate treatment

Disinfection of pepper seed infected with capsicum mosaic virus (CaMV) can be immersed in 100 g/L of trisodium phosphate solution for approximately 30 minutes to obtain near-complete virus inactivation; these treatments had no effects on germination.

3.2.5 Seed Protectants

Thiram is the most commonly used seed-protectant fungicides for vegetable crops~~.~~ Capsicum seeds treated with thiram or captan at 5 and 2 gm/kg seed, respectively. Pepper and many vegetable seeds were treated with thiram and fludioxonil at the rate of 3.0 and 0.05-0.10 ml/kg seed to protect from dampling-off (Lamichhane et.al.2020).

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