



GENERAL GUIDELINES

Seed Health

Fostering the safe distribution of maize and wheat seed

Fourth edition

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Monica Mezzalama



CIMMYT – the International Maize and Wheat Improvement Center – is the global leader in publicly-funded maize and wheat research-for-development. Headquartered near Mexico City, CIMMYT works with hundreds of partners worldwide to sustainably increase the productivity of maize and wheat cropping systems, thus improving global food security and reducing poverty. CIMMYT is a member of the CGIAR Consortium and leads the CGIAR Research Programs on MAIZE and WHEAT. The Center receives support from national governments, foundations, development banks and other public and private agencies.

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Fostering the safe distribution of maize and wheat seed: General guidelines

Introduction

CIMMYT has a global mandate for the improvement of wheat and maize, and it is also responsible for conserving the germplasm of these crops. CIMMYT's germplasm improvement programs rely heavily on the free international exchange of maize and wheat seed. All concerned institutions, cooperators contributors and regulating authorities must have confidence in the safety of both imported and exported seed to facilitate such exchange. CIMMYT is fully committed to maintaining fundamental health standards in its worldwide operations. These standards are dictated at different levels by the International Plant Protection Convention (FAO 1997 https://www.ippc.int/en/), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, FAO 2002), and by the CGIAR (SGRP-CGIAR, 2010).

Seed borne or seed associated pathogens and pests can be carried **on** the seed surface (such as fungi and bacteria). in the seed (infecting the internal tissues, fungi bacteria and viruses) and with the seed (insects, nematodes, resting spores of fungi such as bunts, sclerotia of Claviceps spp.). In all cases, regardless of the location on the seed or if the pathogens can be transmitted to the new formed plant directly from the infested seed, there are species of quarantine and economic importance. On many occasions quarantine requirements are obsolete or not scientifically based. That is why it is important for CIMMYT to apply updated standards that will help to prevent any pathogen dissemination around the world. At the same time, it is important to avoid generating alarm among our collaborators and among country phytosanitary authorities. When new information on the presence of a new pathogen or of the increased relevance of an already present pathogen in a certain area is obtained it is always necessary to confirm the source and to contact a local reliable plant pathologist to investigate the situation and evaluate the level of risk involved.

The aim of this document is to provide general guidelines to foster safe germplasm movement from and to CIMMYT offices. These guidelines may contain a lot of "common sense" information that it can be handy to have available and easy to consult. The information related to the country requirements will be updated yearly, and new sources of information and references will also be added.

List of seed borne and seed transmitted pathogens

Tables 1 and 2 report wheat, triticale and maize seed borne and seed transmitted pathogens.

All these can have different relevance for each country in which the seed is produced or for destination countries. Nevertheless, pathogens such as *Tilletia indica* (Karnal bunt) and *Tilletia controversa* (dwarf bunt) of wheat and *Pantoea stewartii* (Stewart's wilt) of maize are the most commonly listed in quarantine regulations. The tables also summarize information regarding geographical distribution (obtained mainly from CABI Crop Protection Compendium online edition 2016), the most used method for detection and the most commonly used treatment.

List of main regulated pathogens worldwide

Tables 3 and 4 report the most updated information on regulated seed borne pathogens of wheat and maize collected at CIMMYT headquarters on the basis of the permits granted to CIMMYT collaborators requesting our germplasm worldwide.

Standard seed production procedures

Field inspections

Field inspections are very useful to detect the presence of insects and pathogens several times at different growing stages of the seed multiplication plots. During these inspections plants showing symptoms of undesirable pathogens should be extirpated or a pesticide treatment should be applied if possible. Field inspections in many countries are the only procedure required for the granting of the International Phytosanitary Certificate. Not all seed borne pathogens can be detected, because many do not show visible symptoms, nevertheless field inspections help to understand the situation of the crop and foresee the quality of the seed that will be produced.

The control of foliar diseases, such as rusts, leaf spots and blotches, mildew, insects etc. help to produce better quality seed, reducing the risk of post-harvest problems.

There are specific guidelines for carrying out proper field inspections:

- Establish a field inspection pattern in order to cover all parts of the field (for example see: National Seed Heath System, USDA-APHIS)
- The crop should be inspected at several growth stages (after germination, 2-4 leaves for wheat and maize; tillering, flowering and before maturity in wheat; every 2 weeks for maize up to tasseling)
- Plants showing symptoms can be taken to a laboratory for analysis and diagnosis

Harvest procedures

Harvest has a very important role in maintaining the quality of the seed. Right maturity, good and well calibrated equipment are of the utmost importance to conserve the integrity of the seed in the post-harvest step. Moisture content higher than 13-14% favors seed pathogens, reducing viability and vigor. Seed longevity, important for seed devoted to long term storage, is also affected by the moisture content at harvest, as it becomes shorter the higher the moisture content.

It is very important also to remove the seed from the field and to not leave the harvested seed in the open air too long to avoid aerial contaminations.

Storehouse maintenance

Storehouses can be cleaned regularly by washing surfaces with water sodium chloride solution (1%) or with a mild soap water solution, in particular before storing new harvested seed. In case of insect infestation, fumigation with phosphine every 2-3 months can be carried out: the doses per m³ are usually indicated on the product label; the storehouse must be kept tightly closed for 2-3 days and then it must be aerated before allowing people to go in.

Standard seed preparation procedures

Seed testing

Plant or seed samples should be taken to a laboratory facility with recognized proficiency in diagnosing plant diseases (for example with International Seed Testing Association (ISTA) or International Organization for Standardization (ISO) accreditations). Seed for testing must be sampled according to ISTA protocols or instructions given by the testing laboratory in order to obtain reliable results. Seed can be tested for fungi, bacteria, viruses, phytoplasma and nematodes depending on the need. The testing can be focused on the pathogens of quarantine importance and depending on the results you can determine whether the seed is suitable for shipping or not. Seed can be also tested for viability and vigor if necessary.

Testing for the unintentional presence of GMOs

CIMMYT has guidelines for the detection of unintentional presence of transgenes in maize produced in the experimental stations in Mexico and coming from countries that grow GM maize in open field. (see CIMMYT Intranet site at statement-on-genetically-modified-crop-varieties and research-services/policies-and-guidelines). CIMMYT HQ outsources the service with an internationally recognized laboratory. The same type of service can be requested by regional offices.

Seed treatment

Treatment can be applied to seed with different purposes:

- Seed disinfection = elimination of a pathogen that penetrated into living cells of the seed and became established
- Seed disinfestation = control of spores or other forms of pathogenic organisms found on the surface of the seed
- Seed protection = protect the seed and young seedling from pathogenic organisms in soil

According to this and to the target pathogens different active ingredients can be chosen (see tables 1 and 2). The most recommended way of seed applications is as slurry, which allows several active ingredients to be mixed and applied at one time. A slurry can be found commercially or obtained by mixing water with the powder compound. When the treatment is required, but no specific compound is mentioned on the import permit, wide spectrum fungicides and insecticides can be used. An effective disinfection of the seed surface can be obtained by washing seed for 3 minutes in a water solution of NaClO at 1.2% and rinsing seed in water: it is advisable to test seed germination when this procedure is applied for the first time. Usually after treatment seed must be dried; ether at 27-28 °C for 2 hours or in open air but being careful to protect the seed from aerial contaminations.

Seed packing

Seed packing must be carried out in a clean closed area, after having disinfected the floor and surfaces either with chlorine water solution (1%) or by washing with a mild soap. New envelopes and boxes should also be used.

Documentation

Shipments of seed for experimental use must be accompanied by a number of documents that are essential to allow the introduction of seed into a country.

The following documents are mandatory for all signatory countries of the IPPC International Phytosanitary Certificate (International Standards for Phytosanitary Measures, ISPM No. 12. Guidelines for Phytosanitary Certificates, 2011, FAO). Generally phytosanitary authorities in all countries are very strict on this document so it is advisable to fill it out with a typewriter or electronically, and to avoid any handmade corrections. Stamps and signatures must be absolutely original (electronic signatures and stamps are usually not accepted unless the country that granted them has informed the phytosanitary authorities of the importing country about their validation procedure).

The Material Transfer Agreement (see CIMMYT website at cimmyt-intellectual-property-policy and policies/cimmytspolicy-on-germplasm- distribution) has become mandatory for the signatories of the ITPGRFA. CIMMYT distributes seed under the Standard Material Transfer Agreement. Seed imported under this or any other kind of agreement must be reviewed by the CIMMYT Intellectual Property office at headquarters.

A certificate of origin, which is usually a simple document issued on institutional letterhead paper declaring: species, site, year, weight, use of the seed and signature of the person responsible of the distribution.

A commercial invoice that declares that the seed has no commercial value, its species and weight. In some countries declaring zero maybe a problem for customs, so a symbolic value can be given such as \$5 USD.

Not always required:

The GMO free declaration. The terms of this declaration can be different depending on the situation of transgenic crops in the country of origin of the seed (presence in open field, experimental etc.). At headquarters (HQ) the declaration is signed by the Director General (DG) or the Deputy Director General (DDG), in regional offices it should be signed by the CIMMYT Country Representative (CRR).

Glossary

DGSV Mexican Phytosanitary Authority (Spanish acronym)

GMO = Genetically Modified Organism

IPPC = International Plant Protection Convention ISO International Organization for Standardization

= International Seed Testing Association **ISTA** ITPGRFA = International Treaty on Plant Genetic Resources for Food and Agriculture

KΒ = Karnal bunt

SHL Seed Health Laboratory

Table 1. Wheat and triticale pathogens carried by seed, with information on seed transmission levels, references, geographical distribution, seed testing procedure, and recommended seed treatment

Pathogen	Common name	Seed Transmission	Distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi. org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Barley Stripe Mosaic Virus	Stripe mosaic of barley	Transmissibility (up to 90-100%) depends on the virus strain as well as on the stage of development at which plants become infected, and it is influenced by temperature. The percentage of pollen and ovule transmission varies from 10 to 35% and from 17 to 66%, respectively (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).		ELISA*	None available
Wheat Streak Mosaic Virus	Wheat streak	Transmission ranges from 0.2 to 1.5%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. Roger A.A. et al. 2005. <i>Plant Disease</i> 89: 1048-1050).	Worldwide	ELISA* RT-PCR**	None available
Pseudomonas fuscovaginae	Bacterial sheath rot Sheath brown rot	Transmission proven but level not established. (Duveiller, E., and C. Martinez. 1990. Seed detection of <i>Pseudomonas fuscovaginae</i> in wheat. <i>Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent</i> 55(3a): 1047-1053).	South-East Asia, Central and South America, Europe, Africa	Cultural, biochemical methods. Multiplex PCR	Kasugamycin (partially effective)
Pseudomonas syringae pv. atrofaciens	Basal glume rot	Transmission proven but level not established. (Duveiller, E., L. Fucikovsky, and K. Rudolph (eds.). 1997. The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, D.F.: CIMMYT. p. 56.)	Iran Morocco South Africa Zimbabwe Canada USA Italy Eastern Europe Australia New Zealand	Agar plate method on KBC medium	None available
Rathayibacter tritici	Spike blight Tundu disease	The pathogen is seed borne, it is carried with the seed in infected galls of <i>Anguina tritici</i> . (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc.).	Cyprus, Afghanistan, China, India, Iran, Iraq, Pakistan, Egypt, Ethiopia, Morocco, Zambia, Australia		None available Elimination of the nematode galls.
Xanthomonas translucens pv. undulosa	Wheat black chaff	Transmitted at up to 25%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc.)	Asia North America Europe Brazil Australia	Agar plate method on Wilbrink's medium PCR	None available
Alternaria triticina	Leaf blight of wheat Alternaria Leaf Blight	Seed-borne inoculum plays a major role in disease perpetuation, but level of transmission not established. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Asia Egypt, Nigeria Mexico, Argentina France, Greece, Italy, Macedonia, Portugal, Russian Federation	Agar plate method on selective medium Freezing blotter test	Carboxin Mancozeb Iprodione Thiram Triazole fungicides

^{*} ELISA= Enzyme linked immunosorbent assay

^{**} RT-PCR = Reverse transcription polymerase chain reaction

Pathogen	Common name	Seed Transmission	Distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi. org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Hymenula cerealis	Cephalosporium stripe	Transmission between 0.18 and 0.55%. (Murray, T.D. 2006. <i>Plant Disease</i> 90: 803-806).	India, Japan, Republic of Korea, Egypt, South Africa, Canada, Mexico, USA, Dominican Republic, Austria, Denmark, Germany, Italy, Netherlands, Poland, Sweden, UK	Freezing blotter test PCR	None available
Claviceps purpurea	Ergot	The pathogen is seedborne, not seed transmitted. Up to 70% of the seed can be replaced by sclerotia. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Worldwide	Visual inspection	None available (physical removal of sclerotia)
Cochliobolus sativus Bipolaris sorokiniana	Common root and crown rot Spot blotch Kernel black point	Transmitted up to 90%. (Goulart, A.C.P. 1996. <i>Summa</i> <i>Phytopathologica</i> 22(1): 5-9.)	Worldwide. Common root rot is mainly found in dryland areas whereas spot blotch occurs in warmer and humid environments.	Freezing blotter test	Difenoconazole Tebuconazole
Gibberella avenacea	Fusarium blight	Transmission from seed to seedlings proven under laboratory conditions. (Porta-Puglia, A., and S. Santorelli. 1994. Sementi Elette 40(5): 35-38.)	Worldwide	Freezing blotter test	Fludioxonil Triazole group
Gibberella zeae	Fusarium blight	Transmission from 55 to 94%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc).	Worldwide	Freezing blotter test	Fludioxonil Triazole group
Magnaporthe oryzae Triticum pathotype	Wheat blast	Different levels of transmission depending on environmental conditions at sowing. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. (Urashima, A.S., et al. 2009. Advances in Genetics, Genomics, and Control of Rice Blast Disease. Dordrecht: Springer Netherlands. pp. 267-277).	Northern Argentina, Bolivia, Brazil, Paraguay Bangladesh	Freezing blotter test	Benomyl Carboxin Difenoconazole Tebuconazole Thiofanate - Metyl Thiram
Monographella nivalis	Pink snow mold Seedling blight of cereals Snow blight of cereals Snow mold of cereals	Rate of transmission unclear, although seed-borne inoculum is the main cause of seedling blight and reduced germination. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Worldwide	Freezing blotter test	Triadimenol, Bitertanol, Prothioconazole Strobilurins (e.g. Fluoxastobin).
Phaeosphaeria nodorum	Stagonospora glume blotch	The relationship between seed infection and disease incidence on upper leaves is not linear, but in some cases transmission can reach 40%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Worldwide	Freezing blotter test	Prochloraz, Triazoles, Carbendazim
Pyrenophora tritici- repentis (Helminthosporium tritici-repentis)	Tan spot	Seed transmission at up to 92% in vitro and 60% in potting soil outdoors. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Worldwide	Freezing blotter test	Difenoconazole, Thiram, Triticonazole, Carboxin

Pathogen Sclerophthora macrospora	Common name Downy mildew	Seed Transmission Evidence of transmission under laboratory conditions only. (Bains, S.S., and J.S. Jhooty. 1985. Seed Research 13(2): 154-156.)	Distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi. org/cpc) Australia Austria, Bulgaria, Canada China, Ethiopia, Germany, India, Iran, Iraq, Italy, Japan, Korea, Mauritius, Mexico, New Zealand, Pakistan,	Seed testing procedure Staining of seed tissue	Recommended seed treatment (active ingredient) None available
Tilletia controversa	Dwarf bunt	Seed borne but not seed transmitted; bunted	Peru, Poland, South Africa, Syria, Taiwan, Turkey, Uganda, USA (Arizona, Arkansas, North Dakota), former USSR, former Yugoslavia Afghanistan, Armenia, Georgia	Seed washing and	Difenoconazole
Timeta controversa	Swart State	grains release spores into soil, where they can germinate and infect new seedlings immediately after emergence. (Grey, W.E., et al. 1986. <i>Plant Disease</i> 70(2): 122-125. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, D.F.: CIMMYT.)	Rightinistan, Amenia, deorgia (Republic of), Iraq, Japan, Kazakhstan, Kyrgyzstan, Syria, Tajikistan, Turkey, Turkmenistan, Uzbekistan, Algeria, Libya, Tunisia, Canada, Usa, Argentina, Albania,, Austria, Bulgaria, Croatia, Czech Republic, Germany, Greece, Hungary, Italy, Latvia, Luxembourg, Moldova, Montenegro, Poland, Romania, Russian Federation, Slovakia, Slovenia, Sweden, Switzerland, Ukraine	filtration or centrifugation test	BIIOTOGOTAZOG
Tilletia indica	Kamal bunt	Seed borne but not seed transmitted; bunted grains release spores into soil, where they can germinate and infect plants at the flowering stage. (Wilcoxson, R.D., and E.E. Saari (eds.). 1996. Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, D.F.: CIMMYT).	Afghanistan, India, Iran, Iraq, Nepal, Pakistan, South Africa, Mexico, USA	Visual inspection NaOH seed soaking Seed washing and centrifugation or filtration PCR (ISPM 27 Diagnostic protocols for regulated pests DP 4: Tilletia indica Mitra. Adopted 2014; published 2016. International Plant Protection Convention https://www.ippc.int/ en/)	Seed washing with 1.3% NaClO solution for 3 minutes Chlorothalonil
Tilletia tritici Tilletia laevis (=T. foetida)	Common bunt	Seed borne but not seed transmitted; bunted grains release spores into soil where they can germinate and infect new seedlings before emergence. (Wilcoxson, R.D., and E.E. Saari (eds.). 1996. Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management. Mexico, D.F.: CIMMYT.)	Worldwide	Visual inspection Seed washing and filtration	Fluoxastrobin Benzimidazole group Carboxin Triazole

Pathogen	Common name	Seed Transmission	Distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi. org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Urocystis agropyri	Flag smut of wheat	Seed borne but not seed transmitted; bunted grains release spores into soil, spores released in soil infect coleoptile before leaf emergence. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc).	Afghanistan, China, India, Iran, Iraq, Israel, Japan, Kazakhstan, Korea, DPR, Korea, Republic of, Mongolia, Nepal, Pakistan, Taiwan, Turkey, Uzbekistan, Algeria, Egypt, Libya, Morocco, South Africa, Tunisia, Canada, Mexico, USA, Guatemala, Argentina, Bolivia, Chile, Uruguay, Venezuela, Baltic States, Bulgaria, Cyprus, Czech Republic, Czechoslovakia (former), Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Spain, Sweden, Switzerland, UK, Ukraine, Australia, New Zealand	Field inspections Seed washing and centrifugation or filtration	Carboxin Tebuconazole Triadimenol
Ustilago nuda f.sp. tritici	Loose smut of wheat	A 100% correlation has been found between infected seed and incidence of loose smut in the field. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc).	Worldwide	Embryo test	Carboxin Difenoconazole
Anguina tritici	Wheat seed gall nematode	Seed borne but not seed transmitted; the principal means of dispersion is by wheat seed containing galls. (Luc, M. et al. 1990. Nematode parasites of cereals. In: <i>Plant Parasitic Nematodes in Subtropical and Tropical Agriculture.</i> Wallingford, UK: CAB International. pp. 109-136).	Afghanistan, Azerbaijan, China, India, Iran, Iraq, Israel, Korea Republic of, Pakistan, Saudi Arabia, Syria, Taiwan, Turkey, Egypt, Ethiopia, Austria, Bulgaria, Croatia, Cyprus, France, Germany, Greece, Hungary, Ireland, Italy, Netherlands, Poland, Romania, Russian Federation, Serbia, Slovenia, Spain, Sweden, Switzerland, UK, Ukraine, Serbia, Montenegro, Australia, New Zealand	Visual inspection Seed soaking Separation of the nematode galls from the seed	None available

Pathogen	Common name	Seed Transmission	Distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi. org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Heterodera avenae	Cereal cyst nematode	Seed borne but not seed transmitted; cysts borne externally among grains. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Belgium, Bosnia and Herzegovina, Bulgaria Czech Republic Denmark, Estonia, Former Yugoslavia, France, Germany, Greece, Ireland, Italy, Latvia, Malta, Netherlands, Norway, Poland, Portugal, Russian Federation, Serbia, Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, United Kingdom, China, India, Iran, Israel, Japan, Kazakhstan, Pakistan, Saudi Arabia, Turkey, Algeria, Libya, Morocco, South Africa, Tunisia, Canada, USA, Peru, Australia, New Zealand	Visual inspection, cysts are visible under light microscope	None available

^{*} ELISA= Enzyme linked immunosorbent assay

^{**} RT-PCR = Reverse transcription polymerase chain reaction

Table 2. Maize pathogens carried by seed, with information on seed transmission levels, references, geographical distribution, seed testing procedure, and recommended seed treatment

Pathogen	Common name	Transmission	Geographical distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
High Plains Virus	High plains disease	Transmitted at a very low level (0.008%) in sweet corn. (Forster RL, et al. 2001. Plant Disease, 85(7):696-699).	USA, Australia (only some areas of western Australia)	ELISA*	None available
Maize Chlorotic Mottle Virus	Maize mottle virus	Transmitted at 0.04%. (Jensen S. et al. 1991. Plant Disease 75, 497-498)	China, Taiwan, Thailand, USA, Mexico, Argentina, Brazil, Peru,, Kenya, Congo, Ethiopia, Rwanda, South Sudan, Tanzania, Uganda (Mahuku G. et al. 2015. Phytopathology, 105, 956-965)	Grow out test in sterile soil ELISA* RT-PCR**	Seed treatment with insecticide to control vectors
Maize Dwarf Mosaic Virus	Dwarf mosaic of maize	Transmission at 0.5-2.5%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press 150 pp. Shepherd, R.J. et al. 1965. <i>Plant Disease Reporter</i> 49: 468-469).	Worldwide	Grow out test in sterile soil ELISA* RT-PR**	None available
Sugarcane mosaic virus	Sorghum mosaic	Transmitted at a very low level. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. Mikel, M.A. et al.1984. <i>Phytopathologische Zeitschrift</i> 110(3): 185-191).	Worldwide	Grow out test in sterile soil ELISA* RT-PCR**	None available
Wheat Streak Mosaic Virus	Wheat Streak Mosaic Virus	Transmission at 0.1%. (McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Bulgaria, Croatia, Hungary, Italy, Moldova, Poland, Romania, Russia, Serbia, Montenegro, Slovakia, Ukraine, China, Iran, Jordan, Kazakhstan, Syria, Turkey, Uzbekistan, Canada, Alberta, Manitoba, Ontario, Saskatchewan, Mexico, USA, Argentina, Australia	ELISA* RT-PCR**	None available
Acidovorax avenae subsp. avenae	Bacterial leaf blight	Transmission at 2–4%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc Dange, S.R.S. et al. 1978. Indian Phytopathology 31, 523-524. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Worldwide, though with generally limited distribution	Isolation on selective agar medium Grow out test in sterile soil	None available

 $^{^{\}star}$ ELISA= Enzyme linked immunosorbent assay ** (RT)-PCR = Reverse transcription polymerase chain reaction

Pathogen	Common name	Transmission	Geographical distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Burkholderia andropogonis	Bacterial leaf stripe of sorghum and corn	Not proven to be seed borne or seed transmitted. Only 1 report of seed transmission in dent maize in Venezuela. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc Hernandez, Y., and G. Trujillo. 2001. <i>Interciencia</i> 26(3): 108-112.)	Bulgaria, Hungary, Italy, Poland, Portugal, Russia, Brunei, Hong Kong, China, Iraq, Israel, Honshu, Japan, Pakistan, Philippines, Taiwan, Thailand, Egypt, Ethiopia, Kenya, Nigeria, Rwanda, South Africa, Sudan, Togo, Uganda, Zambia, Zimbabwe, Canada, Mexico; USA, Costa Rica, El Salvador, Haiti, Honduras, Argentina, Brazil, Uruguay, Venezuela, Australia, Federated States of Micronesia, New Zealand		None available
Clavibacter michiganensis subsp. nebraskensis	Goss's bacterial wilt	Transmission at 1.6%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc) Rocheford, T.R. et al. 1985. <i>Phytopathology</i> 75: 1378.	Mid-western USA.	Isolation on CNS medium	None available
Pantoea stewartii	Stewart's wilt	Transmitted at between 0.05% and 30% depending on genotype. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Korea (Republic of), USA, Canada, Mexico, Costa Rica, Puerto Rico, Argentina, Bolivia, Guyana, Peru.	ELISA*	None available
Harpophora maydis (Cephalosporium maydis)	Late wilt	Seed borne up to 11%, but not seed transmitted. Plant infection occurs through soil borne inoculum. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Mohamed, H.A. et al. 1967. Plant Disease Reporter 51, 53-56.	Egypt, Israel*, India, Portugal. *Phytopat. Medit. 2013, 52, 16-29	Freezing blotter test Agar plate method on Potato Dextrose Agar	Carbendazim Captan
Sarocladium strictum (Acremonium strictum)	Acremonium wilt Black bundle disease	Seed borne at up to 40%. Seed transmission recorded (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Czech Republic, Italy, Poland, Russian Federation, Spain, China, India, Iran, Pakistan, Syria, Turkey, Burkina Faso, Cameroon, Egypt, Nigeria, Uganda, USA, Mexico, Argentina, Brasil, Chile	Freezing blotter test Agar plate method on NaCl2% malt extract agar	Benomy, Carbendazim Captan
Claviceps gigantea	Horse's tooth Ergot of maize	soil infect maize ears. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc Munkenvold G.P. and White D.G. 2016. Compendium of Corn Diseases. Fourth Edition. The American Phytopathological Society. St. Paul, Minnesota. 165 p.)	Mexico	Physical inspection of seed	None available
Cochliobolus heterostrophus (Bipolaris maydis)	Southern leaf blight of maize	Race T is seed borne up 99%. Race O seed borne up to 10%. Transmission of race T proven in sterile soil. Race O is not seed transmitted. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.).	Worldwide, predominantly tropics and subtropics. Regions with a warm (20-32°C) and damp growing season are most at risk.	Freezing blotter test	Captan Thiabendazole

Pathogen	Common name		Geographical distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Cochliobolus carbonum	Northern leaf spot of corn Maize leaf spot	Seed borne, not seed transmitted. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Austria, Croatia, Former USSR, France, Greece, Hungary, Italy, Serbia, Montenegro, Switzerland, United Kingdom, Cambodia, China, India, Iran, Iraq, Japan, Sri Lanka, Turkey, Angola, Cameroon, Congo Democratic Republic, Congo, Egypt, Kenya, Malawi, Nigeria, South Africa, Tanzania, Zambia, Zimbabwe, Costa Rica, El Salvador, Guatemala, Honduras, Jamaica, Trinidad and Tobago, Canada, USA, Australia, New Caledonia, New Zealand, Solomon Island, Tonga	Freezing blotter test	Thiram Triadimenol
Gibberella moniliformis	Ear and stalk rot	Seed borne and seed transmitted at up to 90%. (McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Wilke A.L. et al. 2007. Plant Disease, 91,1109-1115)	Worldwide	Freezing blotter test	Captan Difenoconazole Fludioxonil Tebuconazole Thiabendazole
Gibberella zeae	Gibberella ear and stalk rot Headblight of maize	Seed borne up to 66%. Seed transmitted up to 59%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc.)	Worldwide	Freezing blotter test	Captan Difenoconazole Fludioxonil Tebuconazole Thiabendazole
Colletothrichum graminicola (Glomerella graminicola)	Corn anthracnose	Seed borne from 9% up to 50%. Seed transmission depends on the level of seed infection. (McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Warren, H.L. 1977. <i>Phytopathology</i> 67(2): 160-162).	Worldwide	Freezing blotter test	Captan Thiabendazole
Khuskia oryzae (Nigrospora oryzae)	Cob rot of maize	Seed borne up to 13%. Sseed transmission proven. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	China, India, Iran, Nepal, Turkey, Hungary, Poland, Romania, Serbia, Montenegro, India, Egypt, South Africa, Canada, USA, Brasil	Agar plate test	Triazole group
Lasiodiplodia theobromae (Botryodiplodia theobromae)	Black kernel rot	Seed borne up to 50%. Seed transmission not proven in the field. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Visual inspection, Freezing blotter test	None available
Peronoscierospora maydis	Downy mildew of maize Java downy mildew	Seed borne and transmitted only by fresh seed. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	China, India, Indonesia, Japan, Taiwan, Thailand, Congo Democratic Republic, Jamaica, Venezuela, Australia	Embryo examination (aniline blue test)	Metalaxyl

Pathogen	Common name	Transmission	Geographical distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Peronosclerospora philippinensis	Philippine downy mildew	Seed borne up to 70%, but transmitted up to 11% only by fresh seed. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	China, India, Indonesia, Nepal, Pakistan, Philippines, Taiwan, Thailand, Mauritius	Embryo examination (aniline blue test)	Metalaxyl
Peronosclerospora sacchari	Sugarcane downy mildew	Seedborne, and not seed transmitted in seed with less than 20% moisture. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	China, India, Indonesia, Japan, Nepal, Philippines, Taiwan, Thailand, Vietnam, Nigeria, USA, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama, Peru, Australia, Fiji, Papua New Guinea	Embryo examination (aniline blue test)	Metalaxyl
Peronosclerospora sorghi	Mildew of maize and sorghum	Seed borne and seed transmitted also by dry seed up to 12%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Embryo examination (aniline blue test)	Metalaxyl
Phaeocytostroma ambiguum	Phaeocytostroma stalk rot and root rot	Transmission proven under laboratory conditions only. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Australia	Grow out test in sterile soil	Captan
Sclerophthora macrospora	Crazy top of maize Downy mildew	Fresh seed can be infected up to 60%, but this level can go down to 0.6% in dry seed. It is seed transmitted, but 90% of the plants infected are barren, therefore seed transmission is not the main path of pathogen distribution. Plant infection occurs mainly form spores present is soil. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Worldwide	Examination of internal tissues of seed (aniline blue test)	None available
Sclerophthora rayssiae var. zeae	Brown stripe downy mildew	Seed borne and seed transmission lower than 1%. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. Putnam, M.L. 2007. Plant Heath Progress. Online. doi:0.1094/PHP-2007-1108-01-DG. Singh, R.S., et al. 1968. Plant Disease Reporter 52: 446-449.)	India, Myanmar, Nepal, Pakistan, Thailand	Embryo examination (aniline blue test)	Metalaxyl
Setosphaeria turcica	Northern corn leaf blight	Seed borne at a very low level. Transmission suspected but only confirmed by one study. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Worldwide	Freezing blotter test	Carbendazim Mancozeb Fludioxonil Thiram

Pathogen	Common name	Transmission	Geographical distribution (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www. cabi.org/cpc)	Seed testing procedure	Recommended seed treatment (active ingredient)
Setosphaeria rostrata	Rostratum leaf spot	Transmissible, but no data on rate of transmission. (Anahosur, K.H., and A. Sivanesan. 1978. IMI Descriptions of Fungi and Bacteria 59, Sheet 587. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	China, Iran, Pakistan, India, Taiwan, Egypt, Nigeria, USA, Brasil	Freezing blotter test	No information available
Sphacelotheca reiliana	Head smut of maize	Spores are seed-borne on the seed surface, but infection to new plants originates from spores present on the seed surface. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Worldwide	Seed washing, centrifugation and microscopic observation	Triadimenol
Stenocarpella maydis	Ear rot of maize White ear rot of maize	Seed borne and seed transmitted. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Freezing blotter test	Azoxystrobin Fludioxonil Captan Thiabendazole
Ustilago maydis	Common smut of maize	Seed-borne: unattached spores contaminate seed surface, no seed transmission. Main source of inoculum for new plants are spores present in soil released by bunted seed. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/cpc. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.)	Worldwide	Seed washing and filtration	Captan Carboxin Thiabendazole
Heterodera zeae	Corn cyst nematode	Cysts borne externally among grains. (CABI, 2016. Crop Protection Compendium. Wallingford, UK: CAB International. www.cabi.org/ cpc.)	Afghanistan, India, Indonesia, Iran, Iraq, Nepal, Pakistan, Thailand, Egypt, USA, Greece, Portugal	Visual inspection of seed	None available

^{*} ELISA= Enzyme linked immunosorbent assay
** RT-PCR = Reverse transcription polymerase chain reaction

Table 3. List of main regulated wheat pathogens worldwide.

This table contains information collected from the permits granted to CIMMYT Headquarters located in Mexico for exportation of wheat experimental seed. The information received from the same country may vary from a permit to another, therefore the latest has been considered the valid one. The International Phytosanitary certificate is mandatory for all countries, threfore it has not mentioned in the requiremnts for each country.

Before sending a seed shipment it is necessary to contact the consignee in the recipient country to confirm the phytosanitary requirements valid in the country.

The additional declaration "This material is imported under Directive 95/44/EC" applies to all European Community countries. EUROPEAN UNION: (Directive 2000/29/EU) is required for shipments to Europe.

					Dhytoconitony rogui	romente conditros from :
				D. II.		rements, seed free from :
				Pathog	ens	
	Import					
Country	permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes
ALGERIA	Required					
ARGENTINA	Required	Seed must be treated. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica	
AUSTRALIA	Required	Seed must be free of live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem, pod etc), animal material (e.g. faeces, feathers) and any extraneous contamination of quarantine concern.				
AUSTRIA						
BANGLADESH	Required			Rathayibacter tritici	Alternaria triticina, Blumeria graminis, Mycosphaerella graminicola	Anguina tritici, Heterodera avenae
BOLIVIA	Required	The shipment must be free of foreign material (soil and plant residues such as leaves, stems and seeds of other species)			Alternaria triticina, Tilletia indica, Urocystis agropyri	
BOTSWANA	Required				Tilletia indica	
BRAZIL	Required					
BHUTAN	Required	Must be clean and free from soil and other contaminants				
CANADA	Required	Must be clean and free from soil, regulated weed seeds and other regulated pests. The material must be packaged and transported in sturdy leakproof containers.			Tilletia controversa, Tilletia indica, Urocystis agropyri	
CHILE	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	Barley Stripe Mosaic Virus		Tilletia indica	Anguina tritici

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Required		
Trogoderma versicolor, Trogoderma variabile	Cirsium arvense			Fungicide treatment required (product and dose must be specified)	SENASA must be advised of the time, date and place of arrival 48 hours in advance.
					Each shipment must be packed in clean, new packaging, clearly labelled with the full botanical name (i.e. genus and species). Airfreight or mail shipments should have all documentation (e.g. permit or permit number and certification where applicable) securely attached to the outside of the package and clearly marked "Attention Quarantine". Alternatively necessary documentation will need to be presented to AQIS at the time of clearance. Shipments must to be addressed to AQIS as stated in the permit.
			Required		
	Phalaris minor				
Trogoderma variabile, Prostephanus truncatus			Required	Fumigation against insect pests	Must be sent in new containers, packed in a clean and sanitised way that permits phytosanitary inspection.
Prostephanus truncatus, Trogoderma granarium				Consignment must be treated with a specified broad spectrum fungicide seed dressing, OR fumigated with aluminium phosphide within two weeks of sending and accompanied by an original fumigation certificate.	
		Required			
		Required			
				Required	

					Phytosanitary requi	rements, seed free from :
				Pathogens		
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes
CHINA	Required				Alternaria triticina, Tilletia indica, Globisporangium (Pythium) splendens	Ditylenchus dipsaci, s Longidorus spp., Meloidogyne spp., Xiphinema spp., Pratylenchus spp., Trichodorus spp.
COLOMBIA	Required	Must be free from soil, organic material, impurities, and other types of seeds. Must be packed in new envelopes. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	Wheat Streak Mosaic, Barley Stripe Mosaic Virus	Erwinia rhapontici, Rathayibacter tritici, Xanthomonas translucens pv. undulosa Pseudomonas syringae pv. atrofaciens	Alternaria triticina, Colletotrichum graminicola, Dilophospora alopecuri, Fusarium spp., Glomerella graminicola, Helminthosporium spp., Phoma spp., Rynchosporium secalis, Sclerophthora macrospora, Septoria spp., Sphacelotheca cruenta, Tilletia caries, Tilletia controversa, Tilletia indica, Urocystis agropyry, Ustilago spp.	Anguina tritici, Heterodera avenae
CROATIA	Required					
CUBA	Required					
CONGO (Dem. Rep. of)	Required					
ECUADOR	Required	Seed must be treated, fumigated and packed in new envelopes, the seed will be inspected and kept under quarantine at the point of entry. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.				
EGYPT	Required	Thursday (+	+		+
	Required	Must be free from soil, pests and diseases. Freedom from disease as specified by the import permit.			Tilletia indica	
FIJI	Required	Seed must be free from all forms of disease or insect infestation.				
FRANCE	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area				
GERMANY		The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.				
GHANA	Required	The seed is inspected and sampled on its arrival				
GREECE	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.				
GUATEMALA	Required	Certificate of origin, commercial invoice, proof of the arriving shipment (eg. copy of the arwaybill document or similar)		Xanthomonas traslucens pv undulosa		Dythilencus dipsaci
(Rep. of) GUYANA	Required	The seed must be free from soil, trash and quarantine pests				
INDIA	Required	Seed must be free from soil, weeds and plants debris.		Pseudomonas atrofaciens	Claviceps purpurea, Tilletia controversa	
INDONESIA	Required					

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
Bruchus spp., Callosobruchus spp., Diabotrica virgifera, Caulophilus oryzae, Frankliniella occidentalis, Pharaxonotha kirschii, Tribolium destructor, Trogoderma granarium, Trogoderma versicolor, Zabrotes subfasciatus	Ambrosia artemisiifolia, Ambrosia psilostachya, Ambrosia trifida, Ambrosia spp., Cenchrus pauciflorus, Euphorbia dentata, Iva axillaris, Lolium temulentum, Solanum rostratum, Sorghum halepense, Xanthium spp.				
Contarinia tritici, Delia coarctata			Required		
Prostephanus truncatus			Required		
r rostophanus truncatus			rioquirou		
				Seeds must be treated with suitable fungicide and insecticide (5% Malathion/Thiram dust) or its equivalent.	Must be packed in clean, new containers, and must not be packed in hay, straw, chaff etc or any decomposing material.
				Required	
Cryptolestes pusillus, Liposcelis entomophila, Stegobium paniceum,Trogoderma variabile	Cirsium arvense				
				Required	
Sitophilus granarius					

					Phytosanitary requi	rements, seed free from :
				Pathogens		ellichts, seeu nee nom .
	Import			i dulogoni	5	-
Country	permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes
IRAN	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area; it must be free form soil and weeds	Barley Stripe Mosaic	Pseudomonas fuscovaginae Pseudomonas syringae pv. striafaciens Xanthomonas translucens pv. undulosa	Alternaria triticina, Tilletia indica	
IRELAND	Required	Inspection and sampling on arrival		+		
IRAQ	1.64	moposite:	<u> </u>			
ISRAEL	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	Barley Stripe Mosaic Virus, Maize Chlorotic Mottle Virus	Xanthomonas translucens pv. translucens	Tilletia indica, Tilletia controversa, Alternaria triticina, Claviceps purpurea, Sclerophthora macrospora	
ITALY	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica	
JAMAICA	Required	Package and contents must be free from soil, free from injurious insects and diseases and to have been grown in an area free from golden nematode. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	1			Globodera rostochiensis
KENYA	Required	Seed must be clean and free from storage pests.			Tilletia indica, Urocystis agropyri	
LEBANON	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.				
MADAGASCAR	Required	Seed must be treated. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.		Pseudomonas syringae pv. syringae	Sclerophthora macrospora, Tilletia controversa, Tilletia indica, Tilletia laevis, Urocystis agropyri	Ditylenchus dipsaci
MALAWI	Required	Must not contain wild oat seeds. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area	Wheat Streak Mosaic		Urocystis agropyri, Tilletia indica	Anguina tritici
MEXICO	Required	(1)		Pseudomonas syringae pv. atrofaciens	Alternaria triticina, Tilletia controversa, Tilletia indica	Anguina tritici
MONGOLIA	Required	Must be free from pests. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.				
MOZAMBIQUE	Required	Must have been tested at an official laboratory using methods recommended by ISTA, and declared free from quarantine objects and practically free from other harmful organisms. Pesticide use must be in accordance with conditions on Phytosanitary Import License.			Cephalosporium gramineum, Claviceps purpurea, Hymenula cerealis, Tilletia indica, Tilletia controversa, Urocystis agropyri, Ustilago nuda f.sp. tritici	Anguina tritici
NEPAL	Required	Seed must be free from soil, weed seed and plants debris				
NEW CALEDONIA	Required	Must not contain litter, soil, leaves, compost, or any other prohibited organic material.				
NEW ZEALAND	Required					
NIGER	Requierd	Plant and plant parts must be free from soil, inspection on arrival				

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
Trogoderma spp.	Striga spp.		Required	Required	
			Dogwinod		
			Required Required	Required	
			nequireu	nequileu	
			Required		
					Packing material to consist of moss, paper, woodshavings or other artificial media.
	Free from noxious weeds		Required	Required	Banana leaves, maize, rice, sorghum, palm, wheat straw, soil, and leaf mould may not be used as packaging. If any other plant material is used a certificate is required stating: all seeds, pathogens and insects have been killed before use of the material either by heating to 180°F/83°C for 10 minutes or by chemical treatment (details required on IPC).
				Appropriate treatment with fungicide and insecticide.	
Trogoderma granarium, Prostephanus truncatus	Avena fatua		Required	Recommended fungicides and insecticides.	
Marasmia trapezalis, Mayetiola destructor, Sesamia cretica, Sitodiplosis mosellana					
moscialia			Required		
				Required	
				Insecticide and fungicide treatment: triadimenol or carboxine or flutriafol	

			Phytosanitary requirements, seed free from						
				Pathogen					
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes			
NIGERIA	Required								
PAKISTAN	Required	Seed must be treated. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.		Corynebacterium spp., Pseudomonas syringae pv. atrofaciens, Xanthomonas translucens	Tilletia indica, Claviceps purpurea, Ustilago nuda f.sp. trtitici	Anguina tritici			
Papua New Guinea	Required	The crop from which seeds were harvested has been inspected during the growing season and found to be free from quarantine pests and diseases							
PARAGUAY	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	Barley Stripe Mosaic Virus		Tilletia indica, Urocystis agropyri	Anguina tritici, Ditylenchus destructor			
PERU	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.		Pseudomonas syringae pv. syringae, Pseudomonas syringae pv. striafaciens	Tilletia indica, Phaeosphaeria nodorum, Urocystis agropyri				
PHILIPPINES	Required Required	The seed was produced in a Karnal		Erwinia rhapontici	Alternaria triticina, Gibberella avenacea, Sclerospora macrospora, Tilletia tritici, Tilletia laevis Tilletia indica, Tilletia controversa				
		bunt (Tilletia indica) free area.							
PORTUGAL	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica				
ROMANIA	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica				
	Required								
SAUDI ARABIA	Required								
SERBIA	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica				
SOMALIA									
SOUTH AFRICA	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Alternaria triticina, Tilletia controversa, Tilletia indica, Urocystis agropyri, Gloetinia temulenta	Anguina tritici			
SPAIN	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.							
SWEDEN	Required								
SWITZERLAND		The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.			Tilletia indica				
TAJIKISTAN	Required	Must be free of all pests and weeds not present in Tajikistan							
TANZANIA	Required	The seed must be treated. The consignement free from live insects.		Erwinia rhapontici	Cohliobolus lunatus, Tilletia indica, Tilletia tritici	Anguina tritici, Dythilencus dipsaci			
THAILAND	Required	3		Rathayibacter tritici	Alternaria triticina, Blumeria graminis, Septoria tritici	Anguina tritici, Heterodera avenae			
TUNISIA			Barley Stripe Mosaic Virus	Xanthomonas translucens pv. translucens	,	Anguina tritici			

	1				
Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			required		
Trogoderma granarium, Tribolium castaneum			required	Required	
Trogoderma granarium					
Prostephanus truncatus, Spodoptera exigua	Cirsium arvense, Striga spp.		Required	Required	
Liposcelis entomophila, Trogoderma variabile, Trogoderma versicolor				Required	
Sitophilus granarius, Trogoderma variabile					
				Required	
			Required		
Trogoderma granarium	Ambrosia atermisifolia, Ambrosia psilostachya, Ambrosia trifida, Bidens pilosa, Cenchrus pauciflorus, Cuscuta spp., Ipomoea hederacea, Ipomoea lacunosa, Solanum carolinense, Solanum rostratum, Solanum triflorum, Solanum elaeagnifolium		Required		
			Required		
			Required		
			Required		
					Airtight packaging Required.
			Required		
	Phalaris minor				
1	1	1	1	1	

			Phytosanitary requirements, seed free from :				
				Pathogens			
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes	
TURKEY	Required	Non-commercial invoice and list of germplasm required. Seed samples are free from all types of diseases and not transgenic. Statement: Tilletia indica either not found in area grown or not found in observation/testing.			Tilletia indica		
UKRAINE	Required						
UNITED ARAB EMIRATES	Required						
URUGUAY	Required	The seed multiplication plot was officially inspected at least once during growth and found free of [named pathogens] OR The sample is free of official laboratory analysis [laboratory specified].	Barley Stripe Mosaic Virus		Tilletia indica		
UNITED STATES of AMERICA	Required				Tilletia indica, Urocystis agropyri		
UZBEKISTAN	Required	The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.					
ZAMBIA	Required		Barley Yellow Dwarf Virus, Brome Mosaic Virus	Acidovorax avenae subsp. avenae	Gaeumannomyces graminis tritici Tilletia controversa, Tilletia indica, Tilletia tritici, Tilletia laevis, Typhula idahoensis, Urocystis agropyri, Ustilago hordei		
ZIMBABWE	Required	Must be free of live insects and insect eggs. The seed was produced in a Karnal bunt (<i>Tilletia indica</i>) free area.	Barley Stripe Mosaic Virus, Brome Mosaic Virus	Clavibacter iranicus, Clavibacter michiganensis subsp. nebraskensis, Pseudomonas syringae, Rathayibacter iranicus, Xanthomonas translucens	Alternaria triticina, Claviceps purpurea, Giubberella zeae, Monographella nivalis, Pyrenophora seminiperda, Pyrenophora tritici- repentis, Sclerophthora macrospora, Tilletia spp., Urocystis agropyri, Ustilago nuda f.sp. tritici	Anguina tritici	

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
		Required (to be sent with shipment)	Required	Seed washing with NaOCI	
	Cirsium arvense, Lepidium draba, Lolium rigidum, Thlaspi arvense			Insecticide treatment suitable to eliminate Trogoderma variabile (product, concentration, temperature and time must be specified).	
	Free of weed seeds including noxious weeds.			Required	
Trogoderma granarium, Palipsa gularis	Acroptilon repens, Ambrosia trifida, Ambrosia psilostachya, Cenchrus pauciflorusSolanum rostratum, Solanum triflorum				
	Ambrosia artemisifolia, Anagallis arvensis, Cirsium arvense, Cardaria draba, Fumaria officinalis, Lolium temulentum, Melilotus indica, Papaver rhoeas, Phalaris minor, Thlaspi arvense		Required		
Oryzaephilus surinamensis, Prostephanus truncatus, Rhizoperta dominica, Sitophilus granarius, Sitotroga cerealella, Trogoderma granarium, Tribolium castaneum			Required	Required	

Table 4. List of main regulated maize pathogens worldwide.

The table attached contains information collected from the permits granted to CIMMYT Headquarters located in Mexico for exportation of maize experimental seed.

The information received from the same country may vary from a permit to another, therefore the latest has been considered the valid one.

Before sending a shipment contact the consignee in the recipient country to confirm the information reported in this table.

Some of the pathogens listed are not proved to be seed borne (for example *Burkholderia andropogonis, Cercospora zeae-maydis*), nevertheless due to the fact that these pathogens are listed among the country requirements we included them.

Phytosanitary requirement						
				Pathoge		
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes
ALGERIA	Required					
ANGOLA (Rep. of)	Required	Inspection at entrance and sampling				
ARGENTINA	Required			Pantoea stewartii		
AUSTRALIA	Required	Seed must be free of live insects, soil, disease symptoms, prohibited seeds, other plant material (e.g. leaf, stem etc), animal material (e.g. faeces, feathers) and any other extraneous material of quarantine concern.	Wheat Streak Mosaic Virus, Maize Dwarf Mosaic Virus			
BANGLADESH	Required			Pantoea stewartii	Peronosclerospora philippensis, Peronosclerospora sacchari, Peronosclerospora maydis, Sclerophthora rayssiae var. zeae	
BELIZE	Required	Seed must be free of live insects, soil, and diseases of quarantine concern. Seed must be free from weeds				
BENIN	Rquired					
BHUTAN	Required	The material must arrive free of soil, plant residues and weeds.				
BOLIVIA	Required	The shipment must be free of foreign material (soil and plant residues such as leaves, stems and seeds of other species).				
BRAZIL	Required	A composite untreated sample of the content of the shipment must be sent for analysis prior to or together with the shipment. The material is a donation for research purposes in Brazil, has no commercial value.		Pantoea stewartii, Clavibacter michiganensis subsp. nebraskensis	Harpophora (Cepahlosporium) maydis, Kabatiella zeae, Perenosclerospora maydis, Peronosclerospora philippinensis, Peronosclerospora sacchari, Peronosclerospora sorghi, Sclerophthora rayssiae var. zeae, Cercospora sorghi, Mycosphaerella zeae-maydis	Heterodera zeae
CAMBODIA	Required					
CANADA	Required					
CHILE	Required					

	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
				required		
	Diabrotica virgifera virgifera, Trogoderma variabile, Trogoderma versicolor					SENASA must be advised of the time, date and place of arrival 48 hours in advance. Before sending shipment, send by fax the list of materials, invoice letter and Phytosanitary certificate not specified in latest permit
	Trogoderma spp.					Each shipment must be packed in clean, new packaging, clearly labelled with the full botanical name (i.e. genus and species). Airfreight or mail shipments should have all documentation (e.g. permit or permit number and certification where applicable) securely attached to the outside of the package and clearly marked "Attention Quarantine". Alternatively necessary documentation will need to bre presented to AQIS at the time of clearance. Shipments must to be addressed to AQIS as stated in the permit.
				Required		
	Prostephanus truncatus, Trogoderma variabile			Required		Must arrive in new containers and cardboard or wood packaging, packed in a clean and sanitised way that permits phytosanitary inspection.
	Trogoderma granarium, Prostephanus truncatus	Striga spp.	Required			Before sending the shipment the invoice and the list of materials contained in the shipment must be sent by fax to the cooperator.
			required		Ocatoria Oc. 1	
					Carboxin+Captan or Carboxin+Thiram	

			Phytosanitary requirements, seed free from :						
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes			
CHINA	Required	Shipping requirements depend on province. Any requirements are listed on the permit.	Maize Chlorotic Mottle Virus		Peronosclerospora sorghi				
COLOMBIA	Required	See for experimental use/Semilla para uso experimental.		Erwinia chrysanthemi	Peronosclerospora maydis, Peronosclerospora sorghi, Peronosclerospora philippinenensis, Sclerophthora macrospora, Sclerophthora rayssaie var. zeae				
(Rep. Dem. Of) CONGO	Required								
COSTA RICA	Required								
CUBA	Required	Seed inspected and 20% of the seed is sampled on arrival	Cucumber Mosaic Virus, Maize Chlorotic Mottle Virus Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus	Acidovorax aveanae subsp. aevane, Pantoea stewartii, Pseudomonas syringae, Burkholderia andropogonis	Sclerospora graminicola, Sclerophthora macrospora, Stenocarpella macrospora, Stenocarpella maydis	Ditylenchus dipsaci			
ECUADOR	Required			Acidovorax avenae subsp. avenae, Clavibacter michiganenesis nebraskensis, Erwinia chyrsanthemi, Pantoea stewartii, Xanthomonas campestris pv. vasculorum	Claviceps gigantea, Gibberella avenacea, Gibberella moniliformis, Kabatiella zeae, Micospherella pinodes, Micospherella zea-maydis, Pseudoperonospora sorghi, Sarocladium oryzae				
EGYPT	Required	The IPC should indicate variety, description, purity and germination rate of seeds, percentage incidence of weed seeds and their kind and level of seed multiplication. Each package must be printed with the name of the importer, variety, origin and date of expiry.							
EL SALVADOR	Required								
ETHIOPIA (Federal Democratic Rep. of)	Required	Free from soil; the crop must have been inspected during active growth.	Maie Dwarf Virus, Sugarcane Mosaic Virus, Maize Chlorotic Mottle Vrus	Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Peronosclerospora sacchari				
FIJI	Required		Maize Dwarf Mosaic Virus		Ustilago maydis				
FRANCE	Required								

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
Diabrotica virgifera, Prostephanus truncatus	Tribolium dstructor, Ambrosia spp.				
		Required, to be sent at the same time as the shipment	Required		When seed is sent to private companies, an authorization to receive the shipment signed by the manager of the company is required before shipping the seed.
Tenebrio molitor, Trogoderma granarium, Trogoderma variabile	Ageratina adenophora, Agropyron repens, Amaranthus retroflexus, Commelina benghalensis Convolvulus arvensis, Cuscuta spp., Digitaria velutina, Orobanche spp., Pennisetum macrourum, Salvinia molesta, Solanum viarum, Sonchus arvensis				
Atherigona oryzae, Busseola fusca, Caulophilus oryzae, Cryptolestes pusillus, Graphognatus leucoloma, Ostrinia nubilalis, Prostephanus truncatus, Rhyzopertha dominica, Sesamia cretica, Sitophilus zeamays, Spahcelotheca cruenta, Spahcelotheca gingantes, Trogoderma spp.				Fumigation with aluminum phosphide for 72 hours at 3-4 tablets per t and treatment with a protective fungicide	The seed must be sent in new containers.
			Required		
			Required		
Prostephanus truncatus			Required	Fungicide and insecticide always required and must be stated on the International Phytosanitary Certificate	
			Required	Fungicide and insecticide required	

			Phytosanitary requirements, seed				
				ments, seed free from .			
Country	Import permit	Additional requirements	Virus	Pathoger Bacteria	Fungi	Nematodes	
GEORGIA	Required			Pantoea stewartii	Cochliobolus heterostrophus, Stenocarpella macrospora		
GERMANY	Required	Seeds for scientific purpose, or for the purpose of breeding and exhibition.					
GHANA	Required	Seed inspection and sampling on arrival					
GREECE	Required						
GUATEMALA	Required	Certificate of origin and commercial invoice required.			Claviceps gigantea		
HAITI	Required	Inspection on arrival					
INDIA	Required	The seed must be free from soil.	Maize Chlorotic Mottle Virus, Wheat Streak Mosaic Virus	Clavibacter michiganensis subsp. nebraskensis, Pantoea stewartii, Burkholderia andropogonis, Pantoea agglomerans, Pseudomonas fuscovaginae, Pseudomonas syringae pv. coronafaciens	Claviceps gigantea, Cochliobolus heterostrophus, Phakopsora zeae, Kabatiella zeae, Mycosphaerella zeae- maydis		
INDONESIA	Required						
IRAN	Required		High Plain Virus, Maize Chlorotic Mottle Virus	Pantoea stewartii	Glomerella graminicola, Stenocarpella maydis, Claviceps gigantea, Stenocarpella macrospora		
ITALY	Required						
JAMAICA	Required	Package and contents to be free from soil. All plant material to be accompanied by an offical certificate of inspection and of freedom from injurious insects and diseases. The certificate should state that the plants were grown in an area free from golden nematode.				Globodera rostochiensis	
JAPAN	Required			Pantoea stewartii			
KENYA	Required	Plants or plant parts must be entirely free from soil, chaff, and/ or leaf mould. Seed must be treated.	Maize Chlorotic Mottle Virus, Sugarcane Mosaic Virus	Pantoea stewartii	Sclerospora graminicola, Sphacelotheca reiliana, Peronosclerospora sacchari, Ustilago maydis		
KYRGYZSTAN	Required						
MADAGASCAR	Required		Maize Dwarf Mosaic Virus	Pantoea stewartii, Pseudomonas syringae	Peronosclerospora sorghi, Sclerophthora macrospora, Scelrospora graminicola	Ditylenchus dipsaci	
MALAYSIA	Required	The seed must be free of foreign matter, soil and weeds.	Maize Dwarf Mosaic Virus, Maize Streak Virus, Maize Rayado Fino Virus, Corn Stunt (Spiroplasma kunkelii)				

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
Diabrotica virgifera virgifera, Trogoderma granarium					
			Required		The packages or containers (in bundled mixed shipments) are to be specially marked with the name or the company, address of the sender, and species, or mixed shipments may instead be accompanied by appropriate information on species and variety in accompanying documents (precise summary).
Trogoderma variabile	Cirsium arvense				
Prostephanus truncatus, Sitophilus zeamays		Required, to be sent at the same time as the shipment	Required		
Araecerus fasciculatus					
					Packing material to consist of moss, paper, woods havings or other artificial media.
			D : 1		
			Required	Seed to be treated with fungicidal seed dressing before dispatch.	Banana leaves, maize, rice, sorghum, palm, wheat straw, soil, and leaf mould may not be used as packaging. If any other plant material is used certificate is required stating: all seeds, pathogens and insects have bee killed before use of the material either by heating to 180°F/83°C for 10 minutes or by chemical treatment (details required on IPC).

			Phytosanitary requirements, seed free from :						
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes			
MALAWI	Required		Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus, Maize Chlorotic Mottle Virus	Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Drechslera maydis, Kabatiella zeae, Peronosclerospora sacchari, Peronosclerospora philippinensis, Peronosclerospora spontanea				
MAURITIUS	Required	Certified free from dangerous diseases and pests.	Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus, Wheat Streak Mosaic Virus	Clavibacter michiganensis subsp. nebraskensis, Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Claviceps gigantea, Cochliobolus heterostrophus, Drechslera maydis race T, Peronosclerospora philippinensis, Peronosclerospora sacchari, Peronosclerospora sorghi				
MEXICO	Required		Wheat Streak Mosaic Virus	Burkholderia andropogonis, Clavibacter michiganensis subsp. nebraskensis, Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Peronosclerospora sacchari, Sclerophthora rayssiae var. zeae Peronosclerospora philippinensis	Heterodera zeae			
MOZAMBIQUE	Required		Maize Dwarf Mosaic Virus	Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Claviceps gigantea, Kabatiella zeae, Peronosclerospora philippinensis, Peronosclerospora sacchari, Sclerophthora rayssiae var. zeae				
NEPAL	Required								
The NETHERLANDS	Required	Inspection at entrance							
NICARAGUA	Required	Seed is sampled on arrival and tested for GMO	Maize Dwarf Mosaic Virus	Pantoea stewartii	Clviceps gigantes, Gibberella avenacea	Ditylenchus dipsaci			
NIGERIA	Required		Maize Dwarf Mosaic Virus, Maize Streak Virus, Maize Mottle Chlorotic Virus	Clavibacter michiganensis nebraskensis, Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Peronosclerospora maydis, Peronosclerospora sacchari, Peronosclerospora spontanea, Puccini polysora, Sclerophthora macrospora, Ustilago maydis				
PAKISTAN	Required	Must be free from soil and weeds; certified free from insect pests and diseases.		Pantoea stewartii					
PANAMA	Required		Maize Dwarf Mosaic Virus	Pantoea stewartii	Gibberelal zeae, Sclerophthora macrospora, Sclerospora graminicola, Stenocarpella maydis				
PAPUA NEW GUINEA	Required								
PARAGUAY	Required								
PERU	Required			Pantoea stewartii, Pseudomonas syringae pv. syringae	Cochliobolus heterotrophus, Stenocarpella maydis, Stenocarpella macrospora, Sphaceloteca reiliana				
PHILIPPINES	Required	Must be free from soil, plant diseases, insect storage pests and weed seeds. The Plant Quarantine Clearance number from the permit must be stated on the IPC.		Pseudomonas syringae pv. syringae	Claviceps gigantea, Cercospora zeae- maydis				

	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
	Prostephanus truncatus, Trogoderma granarium			Required	Required	The material does not contain any cytoplasmic male sterile lines.
	Prostephanus truncatus				Required (insecticide and fungicide, with description)	Must not be packed in earth, leaf or garden mould, compost, farmyard manure, straw or unsterilized sphagnum moss.
	Busseola fusca, Ostrinia nubilalis					
	Prostephanus truncatus					
	Caulophilus oryzae, Trogoderma variabile			Required		
	Diabotrica virgifera virgifera, Prostephanus truncatus, Trogoderma inlcusum, Listronotus bonariensis			Required		
				Required	Fungicide treatment required (name and dose should be stated on IPC).	
1				Required		
	Trogoderma variabile, Trogoderma versicolor		Required	Required	Always required	List of materials with the number of envelopes and amount of seed per envelope to be sent by fax to cooperator before shipping for confirmatio
	Prostephanus truncatus, Trogoderma variabile			Required	Fungicide and insecticide treatment required (to be stated in IPC)	Seeds should be properly packed.

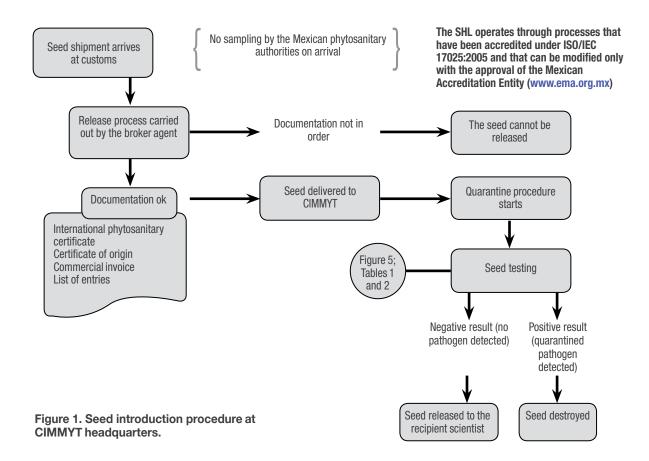
			Phytosanitary requirements, seed free from				
				Pathoge	ns		
Country	Import permit	Additional requirements	Virus	Bacteria	Fungi	Nematodes	
RWANDA	Required	Free from soil tand other debris. Fumigated and treated. Copy of the laboratory results of MCMV, MDMV, SCMV, WSMV	Cucumber Mosaic Virus, Maize Chlorotic Mottle Virus, Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus, Wheat Streak Mosaic Virus	Pantoea stewartii	Cochliobolus heterostrophus, Stenocarpella maydis, Ustilago zeae		
SERBIA	Required						
SIERRA LEONE	Required	Inspected and sampled on arrival					
SPAIN	Required						
SRI LANKA	Required	Inspected and sampled on arrival	Maize Streak Virus, Corn Stunt (<i>Spiroplasma kunkelii</i>)	Clavibacter michiganensis subsp. nebraskensis, Pantoea stewartii	Claviceps gigantea, Harpophora (Cepahlosporium) maydis, Peronosclerosora philippinensis		
SOUTH AFRICA (Rep. of)	Required			Pantoea stewartii	Harpophora (Cepahlosporium) maydis, Cochliobolus heterostrophus, Kabatiella zeae, Peronosclerospora philippinensis, Peronosclerospora sacchari, Peronosclerospora maydis, Sclerophthora rayssiae var. zeae		
SPAIN	Required						
TAJIKISTAN	Required						
TANZANIA	Required		Maize Chlorotic Mottle Virus, Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus	Pantoea stewartii	Peronosclerospora sorghi, Peronosclerospora saccharii, Sclerospora graminicola		
THAILAND	Required	Seed fumigated or treated. Post-entry sampling and testing		Pseudomonas syringae pv. syringae	Claviceps gigantea		
TUNISIA	Required				Harpophora (Cepahlosporium) maydis, Cochliobolus carbonum, Diplodia spp., Gibberella moniliformis, Gibberella zeae		
TURKEY	Required						
UGANDA	Required	Seed must be free from soil, chaff and leaf mold and treated. Original plant import permit and IPC must accompany the seed consignment. Inspection on arrival	Maize Chlorotic Mottle Virus, Maize Dwarf Mosaic Virus		Cochliobolus heterostrophus, Stenocarpella maydis, Ustilago zeae		
UNITED STATES of AMERICA	Required	The requirements and conditions are stated for each exporting country and they may differ depending on the origin of the seed					
URUGUAY	Required			Pantoea stewartii		Aphelenchoides besseyi	
USA	Required						
VIETNAM	Required	Inspection on arrival, treatment and fumigation will be applied					
YEMEN	Required						

Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Required		
			Required		
			nequireu		
Prostephanus truncatus,			Required		
Prostephanus truncatus, Trogoderma granarium, Listronotus bonariensis,					
Chaetocnema pulicaria, Diabotrica virgifera					
			Required	Fungicidal treatment required (details to be stated in IPC)	
Prostephanus truncatus,			Required		
Prostephanus truncatus, Trogoderma granarium, Trogoderma variabile					
			Required		
	Cinaiuma a				
	Cirsium arvense				
	<u>I</u>	1	1	<u> </u>	

					Phytosanitary require	ments, seed free from :
				Pathogei	18	
Country	permit		Virus	Bacteria	Fungi	Nematodes
ZAMBIA	Required	The seed must be treated	Sugarcane Mosaic Virus, Barley Yellow Dwarf Viruses	Acidovorax avenae subsp. avenae, Clavibacter michiganensis subsp. nebraskensis, Pantoea stewartii, Pseudomonas syringae pv. coronafaciens	Claviceps gigantea, Peronosclerospora philippensis, Peronosclerospora sacchari, Sclerophthora macrospora	
ZIMBABWE	Required	Parent plants must have been field inspected during active growth, or specified pathogens must not occur in the state or country of origin, or tested in laboratory and found free of these pathogens. Must be free of live insects and insect eggs.		Pantoea stewartii, Clavibacter michiganenesis subsp. nebraskensis	Helminthosporium maydis race T, Peronosclerospora maydis, Peronosclerospora philippinensis, Peronosclerospora sacchari, Peronosclerospora spontanea, Sclerophthora macrospora, Sclerospora graminicola, Stenocarpella maydis, Ustilago maydis	

	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
		Ambrosia artemisifolia, Anagalis arvensis, Cardaria drada, Cirsium arvense, Fumaria officinalis, Lolium temulentum, Melilotus indica, Papaver rhoeas, Phalaris minor, Raphanus raphanistrum, Spergula arvensis, Thlaspi arvense		Required	Wide spectrum pesticide	
	Prostephanus truncatus, Rhyzopertha dominica			Required	Required	

Seed health procedures for incoming seed



Required documents

Since 25 March 2004 CIMMYT has been recognized by the Mexican government as an international center with international status. Despite this recognition, CIMMYT is not exempt from Mexican phytosanitary laws regarding the import of germplasm into the country.

Mexican authorities require that an authorization be issued to any party (private or public company, international organization, etc.) intending to import seed or vegetative material into Mexico, for any purpose.

CIMMYT must apply for an authorization prior to receipt in Mexico of any maize, wheat, barley or triticale seed. In 2012 CIMMYT and the Mexican Phytosanitary Office, (*Dirección*

General de Sanidad Vegetal, DGSV), branch of the Mexican Ministry of Agriculture), signed an agreement according to which at the beginning of every year CIMMYT must provide a list of countries from where it intends to import seed accompanied by bibliographic references documenting all seed- borne and seed-transmitted pathogens present in the seed's country of origin.

Ordinary import procedure for crop seed entering Mexico

The ordinary seed import authorization issued by Mexico normally takes 3-5 days to be granted, and remains valid as long as the phytosanitary situation in the exporting country does not change and new pathogens are not reported. Once the import authorization is granted, CIMMYT will send a copy to the exporting collaborator. Instructions outlining proper import procedures **must be followed strictly** to ensure successful importation. If any of the required original documentation is missing, the entire shipment will be returned or destroyed without recourse or reimbursement.

The following documents must accompany seed destined for importation to Mexico:

- A copy of the seed import authorization granted by the Mexican government specifically for the country of origin. The original is kept at CIMMYT headquarters;
- The original letter of commercial value;
- The original certificate of origin;
- The original international phytosanitary certificate issued by authorities in the country of origin, including a description of the fungicide used to treat the seed.

Copies of these documents must be included in the box(es) containing the seed. The **originals** should be sent separately but at the same time as the seed shipment. The originals may alternatively be included with the seed, but should be securely placed inside the box to avoid their being lost when the box is inspected at customs. If the original import documents are lost or misplaced before arriving in Mexico, the Mexican authorities will not release the shipment.

Those intending to send seed to CIMMYT should contact their CIMMYT collaborator before doing so for details on the sending procedure.

Special authorizations for importing vegetative material into Mexico

A special import authorization is required in the following cases:

- Seed not supported by international phytosanitary certificate
- Vegetative tissue (leaves, stems, roots, bulbs, etc.)
- Fungal and bacterial cultures or isolates
- DNA and RNA extracts
- Plasmids
- Soil
- Flour, ground seed

Applications for importing the above types of materials must be fully and carefully justified. The application must include the material's precise place and country of origin, its description (variety, weight, origin, and any other useful information), and the aim or purpose of the special importation with a brief description of the research objectives and destination within the country.

Special import authorizations require approximately three weeks of processing time, provided the information submitted is fully satisfactory to the granting authorities. If not, CIMMYT will be asked to provide additional information, and the application process will be restarted. This type of permit is valid ONLY for the specified material. If a shipment is different in content or quantity from that specified on the permit, it will not be released.

Seed health testing

Role of CIMMYT's Seed Health Laboratory (SHL)

The DGSV authorities officially recognize several private and public laboratories in Mexico where seed health testing may be conducted. All imported seed must be checked by one of these laboratories before release, at considerable expense.

Since 1988 the CIMMYT SHL has been officially authorized by DGSV to carry out the quarantine procedures on seed introductions coming into Mexico and CIMMYT, and in April 2007 the SHL obtained accreditation under standard ISO/IEC 17025: 2005, "General requirements for testing and calibration laboratories," as required by the Mexican government. In the SHL three signatories approved by the DGSV, who must renew their accreditation every five years, are the official authorities responsible for seed health testing/diagnostics and for signing the documents that allow the use of the seed after importation or for exportation.

The SHL's official status facilitates seed importation into CIMMYT headquarters in Mexico for experimental purposes, as follows:

- It avoids seed sampling and inspection at the point of entry (e.g., Mexico City airport), since it is done at CIMMYT by the internally approved signatories. This ensures that boxes enter CIMMYT as originally packed and unopened.
- Seed inspection and clearance are expedited.
- It ensures that phytosanitary inspection procedures are meticulously followed.

Laboratory testing

All seed brought into CIMMYT, without exception, **must be subjected to quarantine procedures in the SHL**.

The signatories will check phytosanitary documentation, open the box, and macroscopically inspect the seed for smut sori, nematode galls, ergot sclerotia, weed seeds, insect damage, etc. Based on this inspection and depending on the amount of seed in the shipment, a standardized sampling procedure is applied to obtain the sample to be used for laboratory testing. For details on testing see the section on "Seed health testing procedures at headquarters" (p. 47).

Consignees are informed that the SHL has received the seed with an e-mail report that includes a description of the shipment (arrival date, consignee, origin, list of entries, number of entries, weight, purpose, expected date of release, SHL registration number).

Wheat pathogens considered of quarantine interest in Mexico are listed in the standard *Norma Oficial Mexicana* NOM-017-FITO-1995, and maize pathogens in the *Norma Oficial Mexicana* NOM-018-FITO-1995, established by Mexico's Ministry of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA) and in an updated list of regulated pathogens on the International Plant Protection Convention site (https://www.ippc.int/en/). CIMMYT also has the responsibility to prevent the importation of any other organism considered potentially harmful to these crops by SHL staff (Tables 1 and 2).

The SHL also ensures that shipments are free of weed species, regulated by *Norma Oficial Mexicana* NOM-043-FITO-1999, and from Khapra beetle (*Trogoderma granarium*), regulated by *Norma Oficial Mexicana* NOM-005-FITO-1995.

Results

The SHL signatories judge according to the regulations in force in Mexico whether or not a shipment should be approved for release on the basis of test results. It will be **released** if:

- The seed is pathogen-free, or
- The seed is free of pathogens of quarantine concern in Mexico but contains other undesirable pathogens (Tables 1 and 2). In this case, the SHL will recommend appropriate treatment, to be applied before planting if available; otherwise, the infected seed is destroyed.

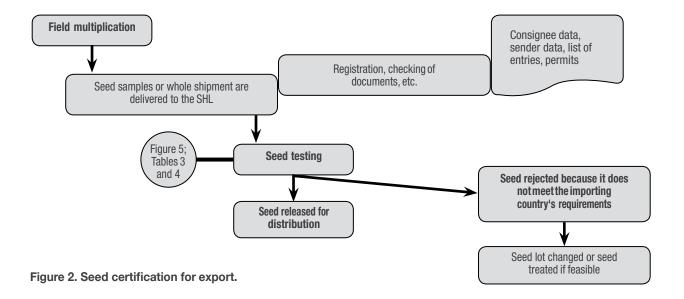
In both cases the SHL sends a notice of release to the consignee.

It will be rejected if:

 The seed carries any pathogen quarantined by Mexico's plant health authorities. In this case the seed will be handled according to the Mexican phytosanitary authorities' instructions. For example, it may be destroyed through incineration or treated.

Every 3 months the SHL must send DSGV authorities a report of all seed introductions arriving at CIMMYT and an update on seed introduction releases.

Seed health procedures for outgoing seed



Required documents

All maize, wheat, and triticale germplasm exported by CIMMYT either in an international nursery or a miscellaneous shipment must be accompanied by the following documents:

- An international phytosanitary certificate issued by the Mexican phytosanitary authorities
- Declaration of value
- A GMO-free declaration when required by importing country
- Optional upon request of the collaborator: a copy of the seed testing report

Certification by CIMMYT is based on examination of samples of all seed and the use of tests conducted by the SHL including seed washing and filtration, greenhouse tests, incubation, and serological and molecular tests.

CIMMYT maize, wheat, and triticale seed is dispatched as clean as possible, to avoid the spread of seed-borne plant diseases and rejection of the seed shipment on arrival in the country of destination. When countries with very strict import regulations require an untreated seed sample, this is sent separately for testing on arrival.

All CIMMYT maize, wheat, and triticale seed that is shipped must be free from quarantined pests.

For details on the laboratory tests carried out by the SHL to detect possible seed-borne and seed-transmitted pathogens see the section on "Seed health testing procedures at headquarters" (p. 47). Additional tests are conducted upon request if the country of destination has special requirements.

Wheat

Seed multiplication in Karnal bunt-free areas

Tilletia indica Mitra is a fungus that causes Karnal bunt (KB) in bread wheat and, to a lesser extent, durum wheat and triticale. It infects wheat florets and the seed that will develop. Spores from infected seed survive in the soil for up to 5 years.

The spread of this pathogen to disease-free areas occurs through seed movement or through other contaminated plant parts or soil. This pathogen is quarantined by 99% of the countries in the world. The pathogen is present in Mexico in some parts of the state of Sonora, where CIMMYT conducts the winter cycle of the shuttle breeding activities. Therefore, to be able to multiply wheat and

triticale seed suitable for international exchange, the CIMMYT Global Wheat Program and the Wheat Germplasm Bank use fields in the municipality of Mexicali (Baja California), in northwestern Mexico. This area was officially declared free of Karnal bunt (KB) in 1997 and is internationally recognized for multiplying seed intended for export. The CIMMYT experimental station of El Batán, (State of Mexico) has also been declared Karnal bunt free since 2009. The seed multiplication procedure is described in figure 3.

The seed meant for multiplication in Mexicali is planted in El Batan during the summer cycle (May-October), plots are sprayed with the systemic fungicide propiconazole every 10 days from spike emergence to the end of grain filling, to protect the plots from possible aerial contamination or infection by *T. indica*.

At harvest it is washed with a 1.2% sodium hypochlorite solution to destroy any possible contamination present on the seed surface. Seed from each line is sampled by the SHL, and filters from seed washings are examined under a stereo microscope. If no contamination is detected, the seed is treated with a mixture of carboxin + captan (3 g/kg of seed) and chlorothalonil (2 g/kg of seed). The seed is then shipped to Mexicali to be sown and multiplied during the winter cycle (November-May).

During the Mexicali crop cycle, the germplasm is constantly monitored to produce seed of the highest quality. It is sprayed 2-3 times with propiconazole during flowering and with terramycin and fungicides to control other foliar diseases, such as yellow rust.

All sowing and harvesting machinery in Mexicali remains there, i.e., it is never moved to non-certified areas where the disease might be present. Visitors to CIMMYT multiplication plots in Mexicali are required to bring clean clothes and shoes to wear and to wash their vehicles before arrival.

The crop is constantly inspected during the cycle. In addition fungicide applications, weed and pest control measures are also carried out.

The harvested seed is packed in new bags that have been appropriately tagged. The new bags and tags to be used at harvest must be shipped by air to Mexicali. Harvested seed is transported to El Batán in a sealed truck that does not pass through KB-contaminated areas.

To avoid contamination, the truck is vacuumed and washed before being loaded. Before entering the CIMMYT station at El Batán, the truck exterior is washed and vacuumed once again, to get rid of any contaminating pathogens, soil, or debris. The truck is then parked in a restricted area reserved exclusively for international nursery seed.

The inside of the storage area at El Batán is washed with a 1% sodium hypochlorite solution, and a sample of the wash water is taken for analysis to confirm that the area is free of any contaminants. There is zero KB tolerance in all areas and during all procedures conducted to ensure noncontamination.

Lines received in the international nurseries area can then undergo final selection by the program head for inclusion in international trials. The selected lines are cleaned by sifting

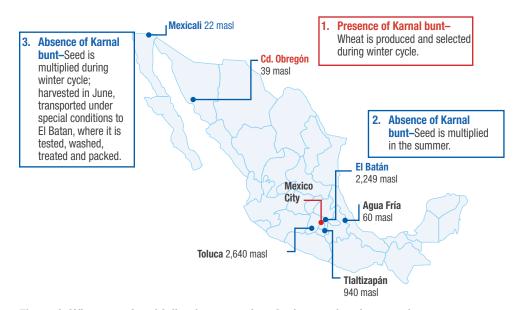


Figure 3. Wheat seed multiplication procedure for international exportaion.

through screens to eliminate weed seed and large debris. Each line is sampled and tested for the presence of viruses, bacteria, and fungi.

Before international distribution, every line of every nursery is also washed using the following procedure:

Washing procedure

The total weight of a line (if exceeding 1 kg) is divided into 800–1000 g portions and placed in perforated metal trays. Trays containing seed are then:

- 1. Rinsed for 3 minutes with water + 10 ml of Tween 20 at high pressure (50-60 pounds/ inch²);
- 2. Immersed for 3 minutes in 1.2% sodium hypochlorite solution; and
- 3. Rinsed for 2 minutes with water at high pressure.

After washing, a further seed sample (of about 50 g) is taken from each tray and again sent to the SHL for a filter test to confirm the absence of contaminating fungal spores.

While the tests are being conducted, the remaining seed, after washing, is treated with a slurry containing chlorothalonil (2 g/kg of seed) and carboxin + captan (3 g/kg of seed), plus an adhesive. The seed is oven dried at 30°C until 9% moisture content is reached. If test results are positive for the presence of any pathogen known to be quarantined, the line is discarded, even if it has already been treated.

Once it is washed, treated, and dried, seed is placed in envelopes, and the nursery is assembled for shipping to CIMMYT partners. Seed selected by visitors during the Yaqui Valley, Sonora cycle must follow this same procedure.

Miscellaneous shipments

"Miscellaneous" shipments contain seed that has not been through a multiplication cycle in Mexicali, that will not be

included in the international nurseries, but which is required to be sent to collaborators as part of research collaborations. However, seed not multiplied in Mexicali merits special care because seed produced in El Batán or Toluca (CIMMYT's highland research station) is of lower quality, mainly due to environmental conditions (high rainfall) and higher disease incidence, and because the crop is not always sprayed with fungicide during the cycle as it is in Mexicali.

Every line is visually inspected to determine the seed's overall condition. When seed quality is very low (5-10% of the seed shows spots or other malformations), the breeder is strongly urged to select the seed for quality before delivering it to the SHL and shipping it to collaborators.

Accurate laboratory examination is required for diseases of quarantine concern. SHL staff decide whether to test every line or make up a composite sample for testing, depending on the amount of seed prepared for shipping. Seed must be washed and chemically treated (as described above) before shipment.

Maize

Maize seed destined for international distribution is produced on CIMMYT's maize research stations in Tlaltizapán (State of Morelos), Agua Fria (State of Puebla) and El Batán (State of Mexico). During the crop cycle, nurseries are periodically inspected in the field, and plants showing symptoms of viral, bacterial and fungal infections are discarded. After harvest, all seed is carefully checked for seed-transmitted pathogens of quarantine interest in countries to which nurseries will be sent.

The germplasm submitted by the Global Maize Program and the Maize Germplasm Bank to the SHL falls into four groups: international nurseries, miscellaneous nurseries, CIMMYT maize lines, and bank accesions. Tests carried out by the SHL on maize seed are discussed in the section on "Seed health testing procedures at headquarters" (p. 47). Maize seed is treated before shipment with a slurry containing: thiametoxam, metalaxil-M, captan, fludioxonil and adherent.

APPENDIX

Guidelines for storing wheat seed under conditions of zero tolerance for Karnal bunt teliospores

Preventive seed health procedures to be applied before placing wheat seed in storage

Warehouse hygiene

Warehouses must be washed and cleaned regularly. Floors and surfaces must be disinfected with a 1.2% sodium hypochlorite solution. To check for contamination, slide traps must be positioned in every chamber, room, and common area and renewed periodically.

Seed testing before storage

Seed to be placed in storage should have been multiplied in areas free of Karnal bunt (KB) and subjected to SHL procedures as follows:

- Seed is tested for viruses, bacteria, and fungi by the SHL.
- To avoid reducing seed viability, seed should not be washed or treated with fungicide. Furthermore, for health reasons, CIMMYT staff should not work with fungicidetreated seed unless absolutely necessary and only if protected by appropriate safety working clothes (gloves, mask, goggles, laboratory coats).
- Seed is rechecked for KB before shipping to collaborators.

Seed is stored in metal or plastic containers to avoid contamination. Upon storage, SHL staff will set up slide traps to detect any contamination.

Preventive measures to be followed by warehouse staff

The number of staff supervising incoming and outgoing materials must be kept to a minimum.

When moving seed or entering the storeroom, staff must wear clothing (aprons or overalls) and footwear reserved specifically for this purpose. Laboratory coats must be washed regularly (every week) even if they have not been worn.

Procedures for cleaning the warehouse after seed is introduced

The storage area must be vacuumed regularly (at least once a week) to get rid of dust. It should never be dusted with a dry cloth. Dust collected in the vacuum bag must be placed very carefully into another bag and burned in an incinerator.

A cloth moistened in a 1% sodium hypochlorite solution may be used to wipe work surfaces. However, it should not be used on metal surfaces that have not been protected from chlorine by anti-corrosion paint (of the kind used in swimming pools).

Rules for moving wheat seed within Mexico from Karnal bunt-contaminated to Karnal bunt-free areas

Mexico's DSGV regulates wheat seed movement within Mexico from areas where KB is present to areas free from the disease, according to the *Norma Oficial Mexicana* NOM- 001-FITO-2001, by granting CIMMYT an authorization that describes the procedure to follow. Therefore:

- All seed shipments must be accompanied by a seed health certificate authorizing seed movement within Mexico (Certificado fitosanitario para la movilización nacional).
- All seed must be washed as described on p. 41.
- All seed must be treated with chlorothalonil.
- All seed produced in the CENEB research station in the Yaqui Valley, Sonora, and arriving at the El Batán or Toluca research stations, must be inspected on arrival by a technician accredited by DSGV.

It should be stressed that seed from the Yaqui Valley may be sown only at El Batán and Toluca.

How to avoid the unintentional spread of *Tilletia indica*.

Tilletia indica Mitra is a fungus that causes Karnal bunt (KB) in bread wheat and, to a lesser extent, durum wheat and triticale. It infects wheat florets and the seed that will develop. Spores from infected seed survive in the soil for up to 5 years.

The spread of this pathogen to free areas occurs through seed movement or through other contaminated plant parts or soil. To avoid this, please observe the following practices:

- No seed or other plant parts or soil can be taken from the field or storehouses.
- Clothes and shoes must be washed when leaving the infested area, and before entering a disease free area.
- Wash (inside and out) vehicles used in the field, before travelling to KB-free areas.
- Wash or disinfect all equipment or instruments used in the field with a 1% chlorine water solution or alcohol before using them in a disease free area.

Guidelines for producing maize seed free from Maize Lethal Necrosis

Since 2011, Maize Lethal Necrosis (MLN) disease has emerged as a major threat to food security in Eastern Africa. MLN is the result of infection of a maize plant by the Maize Chlorotic Mottle Virus (MCMV) and any of the cereal viruses in the Potyviridae group, especially Sugarcane Mosaic Virus (SCMV). Between 2011 and 2014, MLN was reported by most countries in east Africa, including Kenya, Tanzania, Uganda, Rwanda, Democratic Republic of the Congo, and Ethiopia, besides unconfirmed reports of incidence in South Sudan and Burundi. Kenya and Uganda have both registered MLN as a threat to food security in their country reports as per the International Plant Protection Convention (FAO, 1997). The impact of the disease is becoming massive, especially at household level for smallholder farmers who can experience total loss (100 percent of grain yield). MCMV and SCMV infecting a plant individually can be as damaging as MLN.

MLN-causing viruses are transmitted individually in the field from infected maize plants or other co-hosts of MCMV and SCMV by insect-vectors. MCMV and SCMV can also be seed-borne and seed-transmitted. As a consequence, currently, most of the countries importing maize require that the seed is tested and declared free from MCMV and SCMV.

As a general rule, planting of healthy, certified and treated seed is the first step for the production of a healthy crop that can in turn result in healthy seed. If MLN-causing viruses, especially MCMV, enter into a new area through seed, and the infected plants are not diagnosed and rogued out immediately, the control of the disease could become quite difficult. This is due to the possible presence of insect-vectors in the field that can potentially transmit the viruses into neighboring fields. From the phytosanitary perspective, it is important to evaluate the presence of MCMV and SCMV in seed lots meant for exportation to countries where MLN/MCMV is reportedly absent. Ideally, seed produced from a plot that showed MLN-infected plants must NOT be transferred to a known MLN-free location in the same country or outside the country.

Protocol for MLN-free seed production

 Seed treatment: Prior to planting, application of a seed treatment with an insecticide: clothianidin, thiamethoxam, imidacloprid or imidacloprid + thiodicarb, which were demonstrated to provide early stage protection against thrips, aphids and other potential vectors of the MLN pathogens, including beetles (The UK Pesticide Guide, 2015).

• Field management practices:

- Planting plans should be coordinated to account for prevailing winds so that first planted nurseries/trials will be placed as far downwind as possible, and subsequent plantings should progress upwind. This planting scheme minimizes the "Green-Bridge" effect, because insect vectors move from older to younger maize plants and wind direction plays a primary role in vector movement.
- Crop rotation between maize production cycles with a leguminous non-host species is important. The known hosts of SCMV and MCMV include cereal crops (sorghum, oats, millets and sugarcane), common weeds (e.g., Johnson grass), and wild grasses. MCMV incidence is exacerbated in continuous maize production fields (Nelson et al. 2011).
- Maize-free period of at least two months during each calendar year should be pursued as a policy in agreement with local authorities. For this practice to be successful in terms of minimizing MCMV/MLN incidence, it needs to be rigorously enforced. Postharvest monitoring should be performed weekly during that period and maize volunteers destroyed within the farm.
- Timely planting at the onset of the growing season will help reduce disease incidence and pressure that can build up during the season in MLN endemic zones.

Disease control measures:

- Seed production fields must be monitored weekly by trained and certified staff. Rogueing and destruction of plants exhibiting symptoms of viral infection should begin two weeks after planting and should continue until harvest.
- Leaf samples of any symptomatic plants should be delivered to a maize pathologist for diagnostic verification.
- Field inspection records should be maintained, with all the relevant data (date, operator, samples collected, results of testing, number of plants roqued).

- Weed control measures: Agronomic practices to control weed populations must be implemented to control other grass species. As far as possible, grass roadways and walkways should be regularly managed and sprayed with appropriate insecticides to prevent incidence of MLNtransmitting insect vectors.
- Insect vector control measures:
 - Vector monitoring with blue and yellow insect sticky traps placed in a 40 m-grid pattern throughout the nurseries, to effectively monitor the air-borne arrival/ presence of potential insect vectors.
 - Appropriate Insecticides must be sprayed once every 1-2 weeks to control the insect vectors. Multiple active ingredients should be rotated every month during the spray schedule to prevent immunity of target insects to any one insecticide type. Motorized sprayers should be used to uniformly deliver insecticide to all plant parts.
- Field access restrictions: Personnel and occasional visitors must know the procedures to access a seed production site that is maintained MLN-free (e.g., clothing is laundered, no visit of any MLN-infected field prior to entry, providing a brochure to visitors for information on relevant protocol to follow).
- Vehicle and equipment: Vehicles and equipment entering seed production nurseries should be properly cleaned (and interiors vacuumed) prior to entry, if the same vehicle/equipment has been used in other maize fields within the previous 2-3 days.

Leaf sampling for MLN testing

- Leaf sampling is necessary when field inspections are carried out to maintain an MLN-free seed production site. The sample should be taken from the youngest leaf of the plant.
- To carry out the sampling procedure the operator should wear laboratory gloves. A plastic bag must be inverted over one hand. With the inverted plastic bag a portion of the leaf to sample can be grasped. Using the alternate hand, the remaining portion of the leaf must be grasped and torn off into the inverted plastic bag, maintaining the leaf sample inside the bag. The operator must be careful not to touch the interior of the plastic bag with fingers, implements or any other leaves (Figure 4).
- Each sample bag must be labeled with all relevant information (date, site, line, crop stage etc.).
- The labeled leaf samples must be put immediately into a cooler containing freezer blocks. If the samples are not processed immediately they must be refrigerated at 4°C and for no longer than 48 hours. After that time samples will be deteriorated and the results will not be reliable.
- For every symptomatic/infected plant detected, it is advisable to sample at least three neighboring symptom-free plants into separate sampling bags for further analysis. The non-symptomatic plant samples will be indicated as such on the submission label.



Figure 4. Leaf sampling in the field (a-d) for testing of MLN viruses.

Seed sampling for MLN testing

There are no specific guidelines for sampling intensity (number and size of primary samples to be taken from individual seed lots). For experimental seed lots, with a weight lower than 100 kg, an intensity of 10% per entry has been proven to be reliable enough for detection of seedborne virus in maize seed lots produced in MLN endemic countries (CIMMYT Seed Health Laboratory, Mexico). For large amounts of commercial seed, the sampling procedure should be carried out by withdrawing small amounts of seed with a cargo sampler, but being careful to withdraw seed from as many points as possible of the lot. The International Seed Testing Association (http://seedtest.org/en/ productdetail---0--0--24.html), Grain Inspection Packers & Stockyards Administration (http://www.gipsa.usda.gov/ fgis/inspectionservices_testgrading.aspx), and Association of Official Seed Analysts (http://www.aosaseed.com/aasco_ handbook on sampling) provide detailed guidelines for each different seed weight to be sampled.

Protocols for detecting MLN-causing viruses

Both leaf and seed samples can be tested for detecting the possible contamination with MLN-causing viruses using either Enzyme Linked Immunosorbent Assay (ELISA) or Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Both of these methods are reliable, although RT-PCR sensitivity, if the protocol is rigorously followed, can be higher, given that the seed sample tested represents the whole seed lot. It must be noted that seed contains factors that may reduce the sensitivity of the RT-PCR assay. One needs to carefully isolate RNA from the seed first, increasing cost substantially. The choice of a detection method depends on various factors, including availability of financial resources, facilities, reagents, expertise and skills available to carry out the assay, in addition to the level of specificity and sensitivity required, number of samples to be tested, amount of information available on the virus to be detected and the time for completing the test.

For further details, contact: Monica Mezzalama (m. mezzalama@cgiar.org), CIMMYT, Mexico; Biswanath Das (b.das@cgiar.org), Suresh L.M (l.m.suresh@cgiar.org) or B.M. Prasanna (b.m.prasanna@cgiar.org), CIMMYT, Kenya.

Seed health testing procedures at headquarters

All maize, wheat, and triticale seed, both entering and leaving CIMMYT, must pass through the seed health laboratory. All seed undergoes the same set of testing procedures, although the key target pathogens vary between incoming and outgoing seed depending on relevant quarantine regulations.

CIMMYT SHL uses ISO accredited test procedures that are scientifically based on internationally recognized references on seed health (see References).

The tests used in CIMMYT are as follows:

- Physical inspection for smut sori, nematode galls, ergot sclerotia, weed seeds, insect damage, etc.
- Seed wash filter test, which reveals the presence of fungal spores—including bunt teliospores (*Tilletia* spp.), smut spores (*Urocystis* and *Ustilago* spp.), and downy mildew oospores (*Peronosclerospora* and *Sclerophthora* spp.)—and of nematode cysts. This test takes around three hours, although large volumes of samples may take longer. Composite samples of outgoing seed may be used (with rechecking of individual lines in the event of a positive result).

- Freezing blotter test, which reveals the presence of imperfect fungi carried by seed. This test takes two weeks.
- Greenhouse germination test, for the expression, and thus detection, of seed-transmitted pathogens, and to check seed viability. This test takes three weeks. If symptoms appear on seedlings, further testing to identify the causal pathogen is carried out (i.e. ELISA or other tests).
- ELISA, or enzyme-linked immunosorbent assay, to detect specific bacteria and viruses. This test takes 24 hours.
- Seed washing and plating on selective medium for detection of bacteria (Xanthomonas translucens pv. undulosa, Clavibacter michiganensis subsp. nebraskensis).
 This test takes 2 days.
- PCR test for the detection of seed borne bacteria. This test takes 1 day.
- Downy mildew detection test, with microscopic examination of embryo tissues, to detect Peronosclerospora and Sclerophthora spp. on maize. This test takes 24 hours.

A flowchart of the test procedures is given in Figure 5.

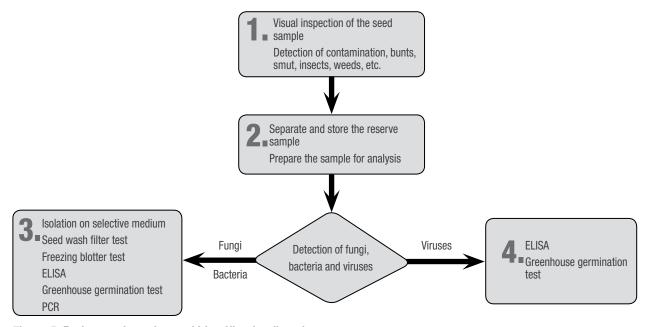


Figure 5. Pathogen detection and identification flowchart.

Procedure for seed wash filter test

This test is for detecting the presence of spores of bunts and smuts, conidia of imperfect fungi, and oospores, either as contaminants or on asymptomatic seed.

Place a 10 or 20 g seed sample in 100 ml of water plus 2-5 drops of Tween 20 and agitate on a shaker for 30 min at 250-300 rpm (if Tween is not available, 1 drop (= $10 \,\mu$ l) of neutral liquid laundry detergent can be used). Filter the wash water through 53 and 15 μ m polyester or nylon meshes. Large spores and debris will remain on the 50 μ m mesh, and *Tilletia indica* teliospores, with an average diameter of

25-40 μ m, will be caught on the 15 μ m mesh. Add a few drops of 3% KOH solution to the mesh and examine under a stereo microscope for the presence of spores. Mark any suspicious structures and confirm their identity under a compound microscope.

Instead of a 15 μ m mesh a Whatman #1 filter paper can be used, placed in a Buchner funnel that is inserted into a flask attached to a vacuum pump. The Whatman #1 filter paper will trap all structures smaller than 50 μ m. Similarly, after filtering, it should be moistened with 3% KOH and observed under a stereo microscope.

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Templates

Letter of commercial value template

[Letterhead of Issuing Institution/Company]

[En papel membretado de la institución o empresa]

Commercial Le	tter
Carta Comerc	
o whom it may concern:	
A quien corresponda:	
Box(es) containing og of (indicate which species) seed samples donate commercial value and an estimated value "for custo	
g de muestras de semilla de (indicar cual especie) donac	
g de muestras de semilla de (indicar cual especie) donac	
g de muestras de semilla de (indicar cual especie) donac	aduanales" de:
Caja(s) que contienen kg de muestras de semilla de (indicar cual especie) donac comercial, con un valor aproximado "solo para propositos	aduanales" de:Shipper's Signature

* Se sugiere la cantidad de US \$0.50 por cada kilo de semilla; no declarar una cantidad total inferior a US

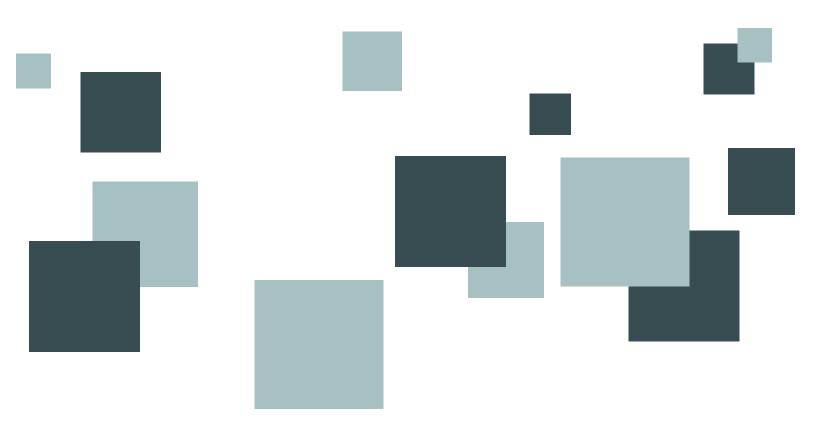
\$1.00 o que exceda US \$100.00.

Certificate of origin template

[On letterhead of shipping institution/company]

(En papel membretado de la institución o empresa)

CERTIFICATE OF ORIGIN
CERTIFICADO DE ORIGEN
To whom it may concern:
A quien corresponda:
Date (Fecha):
DESCRIPTION: Seed of: (indicate the species)
DESCRIPCION: Semilla de: (indicar la especie)
AMOUNT: (in g or kg): CANTIDAD: (en g o kg):
ORIGIN: (Location of production field(s): site, state or province, country)
ORIGEN: (Localización del campo de producción: lugar, estado o provincia, país)
PURPOSE OR USE: Experimental use only
PROPOSITO O USO: Exclusivamente para uso experimental
VALUE: No commercial value
VALOR: Sin ningún valor comercial
REMARKS: Fumigated, treated, etc. (be specific, e.g., Vitavax)
NOTAS: Fumigada, tratada, etc. (especificar, por ejemplo, Vitavax)
AUTHORIZED SIGNATURE:
FIRMA AUTORIZADA:
(Type name and designation of person signing,
and of the shipping institution/company)
(Poner a máquina el nombre y designación del signatario, y de la institución o empresa)





CIMMYT Headquarters Apdo. Postal 6-641 CDMX, Mexico 06600 Tel: +52 (55) 58042004 www.cimmyt.org