

# Seed health



## Fostering the safe distribution of maize and wheat seed

General guidelines

Third edition

Monica Mezzalama

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Headquartered in Mexico, the International Maize and Wheat Improvement Center (known by its Spanish acronym, CIMMYT) is a not-for-profit agriculture research and training organization. The center works to reduce poverty and hunger by sustainably increasing the productivity of maize and wheat in the developing world. CIMMYT maintains the world's largest maize and wheat seed bank and is best known for initiating the Green Revolution, which saved millions of lives across Asia and for which CIMMYT's Dr. Norman Borlaug was awarded the Nobel Peace Prize. CIMMYT is a member of the CGIAR Consortium and 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), by IT-PGRFA (FAO 2002), and by the CGIAR (1999).

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 some 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, new sources of information and references will be also added.

### List of seed borne and seed transmitted pathogens

Table 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 summarize also information regarding geographical distribution (obtained mainly from CABI Crop Protection Compendium 2012 edition), the most used method for detection and the most commonly used treatment. In this section the links to 2 tools developed in the frame of the System-wide Genetic Resources Program ([SGRP](#)) are suggested. These are databases that contain complete information on pathogens and country regulations.

### List of main regulated pathogens worldwide (according to the permits received at HQ)

Table 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 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 (National Seed Health 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 outmost importance to conserve the integrity of the seed in the post-harvest step. It is very important also to remove the seed from the field or do not leave the harvested seed in the open air too long to avoid aerial contaminations.

### **Storehouses maintenance**

Storehouses can be cleaned by washing surfaces with water sodium chloride solution (1%) or with a mild soap water solution regularly 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 m<sup>3</sup> are usually indicated on the product label; the storehouse must be kept tight close for 2-3 days and then it must be aerated before allowing people to get 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 ISTA or ISO accreditations). Seed for testing must be sampled according to ISTA protocols or to 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 GMO**

CIMMYT has guidelines for the detection of unintentional presence of transgenes in maize produced in the experiment stations in Mexico (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 table 1 and 2). The most recommended way of seed applications is as slurry, which can be found commercially as such 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 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

Seed shipments 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 countries:

- The International Phytosanitary Certificate, mandatory among all countries signatories of the IPPC, that also gives instructions about how to fill it (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 with a typewriter or electronically, avoid any handmade corrections, with stamps and signatures absolutely original (electronic signatures and stamps are usually not accepted unless the country that grant them has informed the phytosanitary authorities of the importing country about their validation procedure).

- The Material Transfer Agreement (regarding this please see CIMMYT website at , seed lists, [cimmyt-intellectual-property-policy](#) and [policies/cimmyts-policy-on-germplasm-distribution](#)) which has become mandatory for the signatories of the ITPGRFA. CIMMYT distributes seed under the Standard Material Transfer Agreement and import seed under this or other kind of agreements that it must be reviewed by 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 the no commercial value of the seed, species and weight. In some country declaring zero maybe a problem for custom, 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 HQ the declaration is signed by the DG or the DDG, in regional offices it should be signed by the CLO.

### Glossary

CLO	= Country Liaison Officer
DG	= Director General
DDG-RP	= Deputy Director General for Partnership and Research
GMO	= Genetically Modified Organism
IPPC	= International Plant Protection Convention
ISO	= International Organization for Standardization
ISTA	= International Seed Testing Association
SGRP	= System-wide Genetic Resources Programme
ITPGRFA	= International Treaty on Plant Genetic Resources for Food and Agriculture

**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**  
(visit also [Cropgenebank](#) Safe Transfer of Germplasm (STOG) and Safe Germplasm Movement' (SGM) database)

Pathogen	Common name	Transmission	Distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
Barley Stripe Mosaic Virus	Stripe mosaic of barley	Transmitted at up to 70%. CABI, 2012. Crop Protection Compendium. Wallingford , UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> Maramorosch, K., and K.F. Harris (eds.). 1981. <i>Plant Diseases and Vectors: Ecology and Epidemiology</i> . New York: Academic Press. pp. 293-317.	Worldwide	ELISA*	None available
Wheat Streak Mosaic Virus	Wheat streak mosaic	Transmission ranges from 0.2 to 1.5%. Roger, A.C., B.A. Coutts, A.E. Mackie, and G.I. Dwyer. 2005. Seed transmission of wheat streak mosaic virus shown unequivocally in wheat. <i>Plant Disease</i> 89: 1048-1050.	Bulgaria, Croatia, Hungary, Italy, Moldova, Poland, Romania, Russia, Serbia, Montenegro, Slovakia, Ukraine, China, Xinjiang, Iran, Jordan, Kazakhstan, Syria, Turkey, Uzbekistan, Canada, Mexico, USA, Argentina, Australia	ELISA*	None available
<i>Pseudomonas fuscovaginae</i>	Bacterial sheath 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.	China, Iran, Former Yugoslavia, Russian Federation, Indonesia, Japan, Nepal, Philippines, Burundi, Congo Democratic Republic, Madagascar , Rwanda, Tanzania, Costa Rica, Cuba, Dominican Republic, El Salvador ,Guatemala, Jamaica, Nicaragua Panama Trinidad and Tobago, Mexico, Argentina, Brazil, Bolivia, Chile, Colombia, Ecuador, Peru, Suriname, Uruguay	Cultural and biochemical methods.	None available
<i>Pseudomonas syringae</i> pv. <i>atrofaciens</i>	Basal glume rot	Transmission proven but level not established. Duveiller, E., L. Fucikovsky, and K. Rudolph (eds.). 1997. <i>The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management</i> . Mexico, D.F.: CIMMYT. p. 56.	Worldwide	Agar plate method King's B medium	None available
<i>Rathayibacter tritici</i>	Spike blight (Tundu disease)	The bacterium is carried with the seed in infected galls of <i>Anguina tritici</i> . CABI, 2012. Crop Protection Compendium. Wallingford , UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a>	Cyprus, Afghanistan, China India, Iran, Iraq, Pakistan Egypt , Ethiopia, Morocco, Zambia, Australia	Cultural and biochemical methods.	None available
<i>Xanthomonas translucens</i> pv. <i>undulosa</i>	Wheat black chaff	Transmitted at up to 25%. CAB International. 2007. <i>Crop Protection Compendium, 2007 Edition</i> . Wallingford, UK: CAB International.	Worldwide medium agar plate test	Wilbrink's medium agar plate method	None available

\* ELISA= Enzyme linked immunosorbent assay

Pathogen	Common name	Transmission	Distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Alternaria trititica</i>	Alternaria leaf blight	Seed-borne inoculum plays a major role in disease perpetuation, but level of transmission not established. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Argentina, India, Italy, Mexico, North Africa	Freezing blotter test	Carboxin Mancozeb Iprodione Thiram Soaking seed in water at 52 to 54°C for 10 minutes.
<i>Cephalosporium gramineum</i>	Cephalosporium stripe	Transmission between 0.18 and 0.55%. Murray, T.D. 2006. <i>Seed transmission of Cephalosporium gramineum</i> in winter wheat. <i>Plant Disease</i> 90: 803-806.	Japan, parts of the USA, Dominican Republic, Austria, Germany, Poland, UK, Philippines	Freezing blotter test	None available
<i>Claviceps purpurea</i>	Ergot	Up to 70% of the seed can be replaced by sclerotia. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Worldwide	Visual inspection	None available (physical removal of sclerotia)
<i>Cochliobolus sativus</i>	Common root rot and Leaf spot blotch	Transmitted at up to 90%. Goulart, A.C.P. 1996. Transmission of <i>Bipolaris sorokniana</i> from seeds to wheat coleoptiles. <i>Summa 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. However, there are areas where both diseases can be found. Spot blotch is important in Bangladesh, Bolivia, Brazil, Paraguay, Zambia, Eastern India, and Nepal. Common root rot is severe in Australia, parts of Brazil, parts of Kazakhstan, Canada and the USA	Freezing blotter test	Difenoconazole Tebuconazole
<i>Gibberella avenacea</i>	Fusarium blight	Transmission from seed to seedlings proven under laboratory conditions. Porta-Puglia, A., and S. Santorelli. 1994. Diseases of wheat transmissible by seed. <i>Sementi Elette</i> 40(5): 35-38.	Worldwide	Freezing blotter test	Fludioxonil Triazole group
<i>Gibberella zeae</i>	Fusarium blight	Transmission from 55 to 94%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Worldwide	Freezing blotter test	Fludioxonil Triazole group
<i>Magnaporthe oryzae</i>	Wheat blast	Different levels of transmission depending on environmental conditions at sowing. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Urashima, A.S., C.R.F. Grosso, A. Stabili, E.G. Freitas, C.P. Silva, D.C.S. Netto,	Argentina, Bolivia, Brazil, Paraguay	Freezing blotter test	None available

Pathogen	Common name	Transmission	Distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
		I. Franco, J.H. Merola Botton. 2009. Effect of <i>Magnaporthe grisea</i> on seed germination, yield, and quality of wheat. In G.L. Wang and B. Valent (eds.), <i>Advances in Genetics, Genomics, and Control of Rice Blast Disease</i> . Dordrecht: Springer Netherlands. pp. 267-277.			
<i>Monographella nivalis</i>	Pink snow mold	Rate of transmission unclear, although seed-borne inoculum is the main cause of seedling blight and reduced germination. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Worldwide	Freezing blotter test	Triadimenol, Bitertanol, Prothioconazole, Strobilurins (e.g. Fluoxastrobin).
<i>Phaeosphaeria nodorum</i> ( <i>Stagonospora nodorum</i> )	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, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Worldwide	Freezing blotter test	Prochloraz, Triazoles, Carbendazim
<i>Pyrenophora tritici-repentis</i> ( <i>Helminthosporium tritici-repentis</i> )	Tan spot	Seed transmission at up to 92% in vitro and 60% in potting soil outdoors. Fernandez, M.R., R.M. DePauw, J.M. Clarke, and L.P. Lefkovich. 1996. Red smudge in durum wheat reduces seedling vigour. <i>Canadian Journal of Plant Science</i> 76(2): 321-324	Worldwide	Freezing blotter test	Difenoconazole, Thiram, Triticonazole, Carboxin
<i>Sclerophthora macrospora</i>	Downy mildew	Evidence of transmission under laboratory conditions only. Bains, S.S., and J.S. Jhoo. 1985. Seed transmission of <i>Sclerophthora macrospora</i> in wheat. <i>Seed Research</i> 13(2): 154-156.	Ethiopia, Mauritius, South Africa, Uganda, China, India, Iran, Iraq, Japan, Korea, Pakistan, Taiwan, Turkey, Russian Federation, Australia, New Zealand, Austria, Bulgaria, Italy, Poland, Yugoslavia, Canada, Mexico, USA, Peru	Aniline blue embryo staining method	None available
<i>Tilletia controversa</i>	Dwarf bunt	Seed borne but not seed transmitted; bunted grains release spores into soil, where they can germinate and infect new seedlings immediately after emergence. Grey, W.E., D.E. Mathre, J.A. Hoffmann, R.L. Powelson, and J.A. Fernández. 1986. Importance of seedborne <i>Tilletia controversa</i> for infection of winter wheat and its relationship to international commerce. <i>Plant Disease</i> 70(2): 122-125. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. <i>Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management</i> . Mexico, D.F.: CIMMYT.	Albania, Austria, Bulgaria, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Italy, Lithuania, Luxembourg, Moldova, Poland, Romania, Russian Federation, Serbia, Montenegro, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, Afghanistan, Armenia, Azerbaijan, Georgia (Republic), Iran, Iraq, Japan, Kazakhstan, Kyrgyzstan, Syria, Tajikistan, Turkmenistan, Uzbekistan, Algeria, Libya, Tunisia, Canada, USA, Argentina, Australia	Seed washing and filtration or centrifugation test	Difenoconazole

Pathogen	Common name	Transmission	Distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Tilletia indica</i>	Karnal bunt	Seed borne but not seed transmitted; bunted grains release spores into soil, where they can germinate and infect plants at the flowering stage. Bains, S.S., and H.S. Dhaliwal. 1989. Release of secondary sporidia of <i>Neovossia indica</i> from inoculated wheat spikes. Plant and Soil 115(1): 83-87. Wilcoxson, R.D., and E.E. Saari (eds.). 1996. <i>Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management</i> . Mexico, D.F.: CIMMYT.	Afghanistan, India, Iran, Iraq, Nepal, Pakistan, South Africa, Mexico, USA	Seed washing and filtration or centrifugation test	Seed washing with 1.3% NaClO solution for 3 minutes
<i>Tilletia tritici</i> <i>Tilletia laevis</i> (= <i>T. foetida</i> )	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. <i>Bunt and Smut Diseases of Wheat: Concepts and Methods of Disease Management</i> . Mexico, D.F.: CIMMYT.	Worldwide	Seed washing and filtration or centrifugation test	Benzimidazole group Carboxin Tebuconazole
<i>Urocystis agropyri</i>	Flag smut	Seed borne but not seed transmitted; bunted grains release spores into soil, spores released in soil infect coleoptile before leaf emergence. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Baltic state, Bulgaria, Cyprus, Czechoslovakia (former -), Denmark, Estonia, Finland, France, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russian Federation, Spain, Sweden, Switzerland, Ukraine, United Kingdom, Afghanistan, China, India, Iran, Iraq, Israel, Japan, Kazakhstan, Korea, DPR Korea, Republic of Mongolia, Nepal, Pakistan, Syria, Turkey, Uzbekistan, Algeria, Egypt, Morocco, South Africa, Tunisia, Guatemala, Canada, USA, Australia, New Zealand	Field inspections	Carboxin Cypermethrin Tebuconazole Triadimenol
<i>Ustilago tritici</i>	Loose smut	A 100% correlation has been found between infected seed and incidence of loose smut in the field. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	Worldwide	Seed washing and filtration Embryo test	Carboxin Difencconazole

Pathogen	Common name	Transmission	Distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Anguina tritici</i>	Seed gall nematode (Ear cockles)	Seed borne but not seed transmitted; bunted grains release spores into soil, the principal means of dispersion is by wheat seed containing galls. Luc, M., R.A. Sikora, and J. Bridge. 1990. Nematode parasites of cereals. In: <i>Plant Parasitic Nematodes in Subtropical and Tropical Agriculture</i> . Wallingford, UK: CAB International. pp. 109-136.	Austria, Bulgaria, Croatia, Cyprus, France, Germany, Greece. Hungary, Ireland, Italy, Lithuania, Netherlands, Poland, Romania, Serbia, Montenegro, Spain, Sweden, Switzerland, Ukraine, United Kingdom, Afghanistan, Azerbaijan, China, Taiwan, India, Iran, Iraq, Israel, Korea, Republic of Saudi Arabia, Syria, Turkey, Egypt, Ethiopia, USA, Brazil, Australia, New Zealand	Visual inspection Seed soaking test	None available
<i>Heterodera avenae</i>	Cereal cyst nematode	Seed borne but not seed transmitted; cysts borne externally among grains. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	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	Cysts are visible under light microscope	None available

\* ELISA= Enzyme linked immunosorbent assay

**Table 2. Maize pathogens carried by seed, with information on seed transmission levels, references, geographical distribution, seed testing procedure, and recommended seed treatment**  
(visit also [Cropgenebank](#); Safe Transfer of Germplasm (STOG)) and Safe Germplasm Movement' (SGM) database)

Pathogen	Common name	Transmission	Geographical distribution	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, Seifers DL, Strausbaugh CA, Jensen SG, Ball EM, Harvey TL, 2001. Seed transmission of the High Plains virus in sweet corn. <i>Plant Disease</i> , 85(7):696-699	USA	ELISA*	None available
Maize Chlorotic Mottle Virus	Maize mottle virus	Transmitted at a low level. Delgadillo-Sánchez, F., J.L. Pons-Hernández, and A.D. Torreón-Ibarra. 1994. Seed transmission of maize chlorotic mottle virus. <i>Revista Mexicana de Fitopatología</i> 12: 7-10.	USA, Mexico, Argentina, Brazil, Peru	Soil germination test ELISA*	None available
Maize Dwarf Mosaic Virus	Dwarf mosaic of maize	Transmission at 0.5-2.5%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Shepherd, R.J., and Q.L. Holdeman. 1965. Seed transmission of the Johnsongrass strain of the sugarcane mosaic virus of corn. <i>Plant Disease Reporter</i> 49: 468-469.	Worldwide	Soil germination test ELISA *	None available
Sugarcane Mosaic Virus	Sorghum mosaic	Transmitted at a very low level. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Mikel, M.A., C.J. D'Arcy, and R.E. Ford. 1984. Seed transmission of maize dwarf mosaic virus in sweet corn. <i>Phytopathologische Zeitschrift</i> 110(3): 185-191.	Worldwide	Soil germination test ELISA*	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 and Montenegro, Slovakia, Ukraine, China, Xinjiang, Iran, Jordan, Kazakhstan, Syria, Turkey, Uzbekistan, Canada, Alberta, Manitoba, Ontario, Saskatchewan, Mexico, USA, Argentina, Australia	ELISA*	None available

\* ELISA= Enzyme linked immunosorbent assay

Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Acidovorax avenae</i> subsp. <i>avenae</i>	Bacterial leaf blight	Transmission at 2–4%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Dange, S.R.S., M.M. Payak, and B.L. Renfro. 1978. Seed transmission of <i>Pseudomonas rubrilineans</i> , the incitant of bacterial leaf stripe of maize. <i>Indian Phytopathology</i> 31(4): 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 Germination test on sterile soil	None available
<i>Burkholderia andropogonis</i>	Bacterial leaf stripe of sorghum and corn	Not proven to be seed transmitted. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Elliott, C., and E.F. Smith. 1929. A bacterial stripe disease of sorghum. <i>Journal of Agricultural Research</i> 38: 1-22. Hernandez, Y., and G. Trujillo. 2001. Detection of phytopathogenic bacteria in maize ( <i>Zea mays</i> L.) seeds. <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 and Zimbabwe, Canada Mexico; USA, Costa Rica, El Salvador, Haiti, Honduras, (Argentina; Brazil; Uruguay; Venezuela Australia, Federated States of Micronesia; New Zealand	Isolation on semi-selective agar medium Serology	None available
<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> *	Goss's bacterial wilt	Transmission at 1.6%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Rochefford, T.R., A.K. Vidaver, C.O. Gardner, and D.L. Armbrust. 1985. Effect of wind generated sand abrasion on infection of corn ( <i>Zea mays</i> L.) by <i>Corynebacterium michiganense</i> ssp. <i>nebraskense</i> . (Abstr.). <i>Phytopathology</i> 75: 1378.	Mid-western USA.	Isolation on CNS medium	None available
<i>Pantoea stewartii</i>	Stewart's wilt	Transmitted at between 0.05% and 30% depending on genotype. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Western USA and Canada, Mexico, Costa Rica, Puerto Rico, Bolivia, Brazil, Guyana, Peru, Austria.	ELISA*	None available



Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Acremonium maydis</i>	Late wilt	Transmitted at up to 11%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Mohamed, H.A., W.E. Ashour, A.R. Sirry, and S.M. Fathi. 1967. Fungi carried by corn seed and their importance in causing corn diseases in the United Arab Republic. <i>Plant Disease Reporter</i> 51: 53-56.	Egypt, India.	Freezing blotter test	Carbendazim, Captan
<i>Acremonium strictum</i>	Black bundle disease	Transmitted at up to 40%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Czechoslovakia (former), Italy, Netherlands, Poland, Russian Federation, Spain, China, India, Iran, Pakistan, Syria, Turkey, Egypt, Nigeria, South Africa, USA, Australia	Freezing blotter test	Carbendazim Captan
<i>Claviceps gigantea</i>	Horse's tooth	Seed borne, not seed transmitted. Spores released in soil infect maize ears. White DG, 1999. Ergot. In: Compendium of Corn Diseases. Third Edition (ed. by White DG) APS Press. St. Paul Minnesota, USA. 78 pp.	Mexico	Physical inspection of seed	None available
<i>Cochliobolus heterostrophus</i> ( <i>Bipolaris maydis</i> , <i>Helminthosporium maydis</i> )	Southern leaf blight of maize	Transmitted at up to 99%. 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
<i>Lasiodiplodia theobromae</i> ( <i>Botryodiplodia theobromae</i> )	Black kernel rot	Transmitted at up to 90%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	France, Italy, Romania, Russian Federation, Spain, United Kingdom, Bangladesh, Bhutan, Brunei Darussalam, China, Georgia (Republic), India, Indonesia, Iran, Israel, Japan, Laos, Malaysia, Nepal, Oman, Pakistan Philippines, Singapore, Sri Lanka, Thailand, Turkey, United Arab Emirates, Algeria	Visual inspection, Freezing blotter test	None available

\* ELISA= Enzyme linked immunosorbent assay

Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Cochliobolus carbonum</i>	Northern leaf spot of corn	Seed borne, transmission not proven, but causes problems in seed germination and storage. CABl, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Austria, Bulgaria, Croatia, France, Germany, Greece, Hungary, Italy, Romania, Russian Federation, Serbia and Montenegro, Switzerland, United Kingdom, Cambodia, China, India, Iran, Iraq, Japan, Sri Lanka, 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	Freezing blotter test	Thizam Triadimenol
<i>Fusarium culmorum</i>	Ear rot	Transmitted at up to 38% McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Freezing blotter test	Captan Difenoconazole Fludioxonil Tebuconazole Thiabendazole
<i>Gibberella moniliformis</i>	Ear and stalk rot	Transmitted at up to 100%. CABl, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Freezing blotter test	Captan Difenoconazole Fludioxonil Tebuconazole Thiabendazole
<i>Gibberella zeae</i>	Gibberella ear and stalk rot	Transmitted at up to 60%. CABl, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Foley, D.C. 1983. Effect of symptomless fungal infection of maize seed on germination in the presence and absence of <i>Pythium debaryanum</i> . <i>Proceedings of the Iowa Academy of Science</i> 90(4): 147-149.	Worldwide	Freezing blotter test	Captan Difenoconazole Fludioxonil Tebuconazole Thiabendazole
<i>Glomerella graminicola</i>	Corn anthracnose	Transmitted at from 9% up to 50%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp. Warren, H.L. 1977. Survival of <i>Colletotrichum graminicola</i> in corn kernels. <i>Phytopathology</i> 67(2): 160-162.	Worldwide	Freezing blotter test	Captan Thiabendazole

Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Khushia oryzae</i> ( <i>Nigrospora oryzae</i> )	Cob rot of maize	Transmitted at up to 13%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Hungary, Poland, Romania, Serbia, Montenegro, India, Egypt, South Africa, USA	Agar plate test	Triazole group
<i>Peronosclerospora maydis</i>	Java downy mildew	Transmitted only by fresh seed. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Indonesia, Australia	Microscopic examination of stained seed tissues	Metalaxyl
<i>Peronosclerospora philippinensis</i>	Philippine downy mildew	Transmitted at up to 11% by fresh seed. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	The Philippines, China, India, Indonesia, Nepal, Pakistan, and Thailand	Embryo examination	Metalaxyl
<i>Peronosclerospora sacchari</i>	Sugarcane downy mildew	Transmitted at up to 100%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Australia, Fiji, India, Japan, Nepal, New Guinea, the Philippines, Taiwan, and Thailand	Embryo examination	Metalaxyl
<i>Peronosclerospora sorghi</i>	Mildew of maize and sorghum	Transmitted only by fresh seed. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . 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

Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Phaeocytospora zeae</i>	Phaeocytostroma stalk rot and root rot	Transmission proven under laboratory conditions only. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Australia	Growing out test	Captan
<i>Sclerophthora macrospora</i>	Crazy top downy mildew	Transmitted at from 0.6% up to 60%. McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Ethiopia, Mauritius, South Africa, Uganda, China, India, Iran, Iraq, Japan, Korea, Pakistan, Taiwan, Turkey, Russian Federation, Australia, New Zealand, Austria, Bulgaria, Italy, Poland, Yugoslavia, Canada, Mexico, USA, Peru	Aniline blue embryo staining method	None available
<i>Sclerophthora rayssiae</i> var. <i>zeae</i>	Brown stripe downy mildew	Transmission lower than 1%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . Putnam, M.L. 2007. Brown stripe downy mildew ( <i>Sclerophthora rayssiae</i> var. <i>zeae</i> ) of maize. Online. Plant Health Progress doi: 10.1094/PHP-2007-1108-01-DG. Singh, R.S., M.M. Joshi, and H.S. Chaube. 1968. Further evidence of the seed borne nature of corn downy mildews and their possible control with chemicals. Plant Disease Reporter 52: 446-449.	India, Nepal, Pakistan, Thailand	Embryo examination (aniline blue test)	Metalaxyl
<i>Setosphaeria turcica</i>	Northern corn leaf blight	Transmission suspected but only confirmed by one study. 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
<i>Setosphaeria rostrata</i>	Rostratum leaf spot	Transmissible, but no data on rate of transmission. Anahosur, K.H., and A. Sivanesan. 1978. <i>Setosphaeria rostrata</i> . IMI Descriptions of Fungi and Bacteria 59, Sheet 587.	China, India, Taiwan, Egypt	Freezing blotter test	No information available

Pathogen	Common name	Transmission	Geographical distribution	Seed testing procedure	Recommended seed treatment (active ingredient)
<i>Sphacelotheca reiliana</i>	Head smut	Seed-borne only, but infection can originate from spores present on the seed surface. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Bhutan, China, India, Indonesia, Iran, Iraq, Israel, Japan, Kazakhstan, Korea, Republic of Malaysia, Myanmar, Nepal, Pakistan, Philippines, Taiwan, Yemen, Burkina Faso, Burundi, Cameroon, Chad, Congo, Democratic Republic, Egypt, Eritrea, Ethiopia, Ghana, Kenya, Malawi, Mali, Mauritania, Mauritius, Mozambique, Niger, Nigeria, Rwanda, Senegal, Somalia, South Africa, Sudan, Tanzania, Togo, Uganda, Zambia, Zimbabwe, Canada, Mexico, USA, Barbados, El Salvador, Guatemala, Honduras, Jamaica, Panama, Argentina, Bolivia, Brazil, Colombia, Austria, Bulgaria, Cyprus, Czechoslovakia, (former), France, Germany, Greece, Hungary, Italy, Moldova, Poland, Portugal, Romania, Russian Federation, Spain, Switzerland, Ukraine Yugoslavia (former), Serbia, Montenegro, Australia, New Zealand, Papua, New Guinea	Seed washing, centrifugation and microscopic observation	Triadimenol
<i>Stenocarpella maydis</i>	White ear rot	Transmitted at up to 66.7%. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . McGee, D.C. 1988. Maize Diseases: A Reference Source for Seed Technologists. St. Paul, MN: APS Press. 150 pp.	Worldwide	Freezing blotter test	Captan Thiabendazole
<i>Ustilago maydis</i>	Common smut	Seed-borne but not seed transmitted. CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> . 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 Thiabendazole
<i>Heterodera zeae</i>	Corn cyst nematode	Seed borne but not seed transmitted; bunted grains release spores into soil, cysts borne externally among grains CABI, 2012. Crop Protection Compendium. Wallingford, UK: CAB International. <a href="http://www.cabi.org/cpc">www.cabi.org/cpc</a> .	India, Pakistan, Egypt, USA	Visual inspection of seed	None available

**Table 3. List of main regulated wheat pathogens worldwide.**  
The table attached 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.

Before sending a shipment contact the consignee in the recipient country to confirm the information reported in this table.  
The additional declaration “This material is imported under Directive 95/44/EC” applies to all European Community countries EUROPEAN UNION: (Directive 2000/29/EU).  
You can access this information at the following websites:  
[http://europa.eu.int/eur-lex/en/lif/dat/1995/en\\_395L0044.html](http://europa.eu.int/eur-lex/en/lif/dat/1995/en_395L0044.html) and [http://europa.eu.int/eur-lex/en/lif/dat/2000/en\\_300L0029.html](http://europa.eu.int/eur-lex/en/lif/dat/2000/en_300L0029.html)

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
ALGERIA										Required		
ARGENTINA	Required	Seed must be treated  The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) a free area			<i>Tilletia indica</i>		<i>Trogoderma versicolor</i> , <i>Trogoderma variabile</i>	<i>Cirsium arvense</i>			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.
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.										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.
AUSTRIA										Required		
BANGLADESH	Required			<i>Rathayibacter tritici</i>	<i>Alternaria triticina</i> , <i>Blumeria graminis</i> , <i>Mycosphaerella graminicola</i>	<i>Anguina tritici</i> , <i>Heterodera avenae</i>		<i>Phalaris minor</i>				
BOLIVIA	Required	The shipment must be free of foreign material (soil and plant residues such as leaves, stems and seeds of other species).	Barley Stripe Mosaic Virus		<i>Alternaria triticina</i> , <i>Tilletia indica</i> , <i>Tilletia laevis</i> , <i>Urocystis agropyri</i>		<i>Trogoderma variabile</i> , <i>Prostephanus truncatus</i>			Required	Fumigation against insect pests	Must be sent in new containers, packed in a clean and sanitised way that permits phytosanitary inspection.
BOTSWANA	Required				<i>Tilletia indica</i>		<i>Prostephanus truncatus</i> , <i>Trogoderma granarium</i>				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.	
BRAZIL	Required								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.			<i>Urocystis agropyri</i> , <i>Tilletia controversa</i> , <i>Tilletia indica</i>				Required		Washed with 1.2% hypochlorite solution, treated	
CHILE	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.	Barley Stripe Mosaic Virus		<i>Tilletia indica</i>	<i>Anguina tritici</i>					Required	
CHINA	Required			<i>Pantoea stewartii</i>	<i>Alternaria triticina</i> , <i>Fusarium sacchari</i> , <i>Tilletia indica</i> , <i>Pythium spelndens</i>	<i>Ditylenchus dipsaci</i> , <i>Meloidogyne</i> spp., <i>Xiphinema</i> spp., <i>Pratylenchus</i> spp., <i>Trichodorus</i> spp.	<i>Trogoderma granarium</i> , <i>Trogoderma versicolor</i> , <i>Caulophilus oryzae</i> , <i>Diabotrica virgifera</i> , <i>Frankliniella occidentalis</i> , <i>Pharaxonotha kirschii</i> , <i>Tribolium destructor</i> , <i>Zabrotes subfasciatus</i>	<i>Ambrosia artemisiifolia</i> , <i>Ambrosia psilostachya</i> , <i>Ambrosia trifida</i> , <i>Ambrosia</i> spp., <i>Cenchrus pauciflorus</i> , <i>Euphorbia dentata</i> , <i>Lolium temulentum</i> , <i>Solanum rostratum</i> , <i>Sorghum halepense</i> , <i>Iva axillaris</i> , <i>Tribolium destructor</i> , <i>Xanthium italicum</i>				

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
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	<i>Erwinia rhapontici</i> , <i>Rathayibacter tritici</i> <i>Xanthomonas translucens</i> pv. <i>undulosa</i> <i>Pseudomonas syringae</i> pv. <i>atrofaciens</i>	<i>Alternaria triticina</i> , <i>Colletotrichum graminicola</i> , <i>Lidophia graminis alopecuri</i> , <i>Fusarium</i> spp., <i>Glomerella graminicola</i> , <i>Helminthosporium</i> spp., <i>Phoma</i> spp., <i>Rynchosporium secalis</i> , <i>Sclerophthora macrospora</i> , <i>Septoria</i> spp., <i>Sphacelotheca cruenta</i> , <i>Tilletia caries</i> , <i>Tilletia controversa</i> , <i>Tilletia indica</i> , <i>Urocystis agropyry</i> , <i>Ustilago</i> spp.	<i>Anguina tritici</i> , <i>Heterodera avenae</i>	<i>Contarinia tritici</i> , <i>Delia coarctata</i>			Required		
CROATIA	Required											
CUBA	Required											
CONGO (Dem. Rep. of)	Required											
ECUADOR	Required	Seed must be 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.				Required						
EGYPT	Required											
ETHIOPIA (Federal Democratic Rep. of)	Required	Must be free from soil, pests and diseases.  Freedom from disease as specified by the import permit.			<i>Tilletia indica</i>					Required		
FIJI	Required	Seed must be free from all forms of disease or insect infestation.									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.
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.										
GREECE	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.									Required	
INDIA	Required	Seed must be free from soil, weeds and plants debris.		<i>Pseudomonas atrofaciens</i>	<i>Claviceps purpurea</i> , <i>Tilletia controversa</i>		<i>Sitophilus granarius</i> , <i>Trogoderma</i> spp.					
INDONESIA	Required											
IRAN	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.	Barley Stripe Mosaic Virus, High Plain Virus	<i>Pseudomonas fuscovaginae</i> <i>Pseudomonas syringae</i> pv. <i>striafaciens</i> <i>Xanthomonas translucens</i> pv. <i>undulosa</i>	<i>Alternaria triticina</i> , <i>Tilletia indica</i>		<i>Trogoderma</i> spp.	<i>Striga</i> spp.		Required		
IRAQ										Required		
ISRAEL	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.	Barley Stripe Mosaic Virus, Panicum Mosaic Virus	<i>Xanthomonas translucens</i> pv. <i>translucens</i>	<i>Tilletia indica</i> , <i>Tilletia controversa</i> , <i>Alternaria triticina</i> , <i>Claviceps purpurea</i> , <i>Sclerophthora macrospora</i>					Required	Required	
ITALY	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica</i>					Required		
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.				<i>Globodera rostochiensis</i>						Packing material to consist of moss, paper, woodshavings or other artificial media.

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
KENYA	Required	Seed must be clean and free from storage pests.			<i>Tilletia indica, Urocystis agropyri</i>			Free from noxious weeds		Required	Seed treatment	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).
MADAGASCAR	Required	Seed must be treated.  The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.		<i>Pseudomonas syringae</i> pv <i>syringae</i>	<i>Sclerophthora macrospora, Tilletia controversa, Tilletia indica, Tilletia laevis, Urocystis agropyri</i>	<i>Ditylenchus dipsaci</i>					Appropriate treatment with fungicide and insecticide.	
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		<i>Urocystis agropyri, Tilletia indica</i>	<i>Anguina tritici</i>	<i>Trogoderma granarium</i>			Required	Recommended fungicides and insecticides.	
MEXICO	Required			<i>Pseudomonas syringae</i> pv . <i>atrofaciens</i>	<i>Alternaria triticina, Tilletia controversa, Tilletia indica</i>	<i>Anguina tritici</i>	<i>Marasmia trapezalis, Mayetiola destructor, Sesamia cretica, Sitodiplosis mosellana</i>					
MONGOLIA	Required	Must be free from pests.  The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.								Required		
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.			<i>Cephalosporium gramineum, Claviceps purpurea, Hymenula cerealis, Tilletia indica, Tilletia controversa, Urocystis agropyri, Ustilago nuda</i> f.sp. <i>tritici</i>	<i>Anguina tritici</i>						
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.									Insecticide and fungicide treatment: triadimenol or carboxine or flutriafol.	
NEW ZEALAND	Required											
NIGERIA	Required				<i>Tilletia indica, Urocysitis agropyri</i>	<i>Anguina tritici, Heterodera avenae</i>						
PAKISTAN	Required	Seed must be treated.  The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.		<i>Corynebacterium</i> spp., <i>Pseudomonas syringae</i> pv. <i>atrofaciens</i> , <i>Xanthomonas translucens</i>	<i>Tilletia indica, Claviceps purpurea, Ustilago nuda</i> f.sp. <i>tritici</i>	<i>Anguina tritici</i>	<i>Trogoderma granarium, Tribolium castaneum</i>				Required	
PAPUA NEW GUINEA	Required											
PARAGUAY	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.	Barley Stripe Mosaic Virus		<i>Tilletia indica, Urocystis agropyri</i>	<i>Anguina tritici, Ditylenchus destructor</i>	<i>Prostephanus truncatus, Spodoptera exigua</i>	<i>Cirsium arvense, Striga</i> spp.			Required	
PERU	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.		<i>Pseudomonas syringae</i> pv. <i>syringae</i> , <i>Pseudomonas syringae</i> pv. <i>striafaciens</i>	<i>Tilletia indica, Phaeosphaeria nodorum, Urocystis agropyri</i>		<i>Liposcelis entomophila, Trogoderma variabile, Trogoderma versicolor</i>				Required	
PHILIPPINES	Required			<i>Pectobacterium rhapontici</i>	<i>Alternaria triticina, Gibberella avenacea, Sclerospora macrospora, Tilletia tritici, Tilletia laevis</i>		<i>Sitophilus granarius, Trogoderma variabile</i>					
POLAND	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica, Tilletia controversa</i>						Required	



			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
PORTUGAL	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica</i>							
ROMANIA	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica</i>					Required		
RUSSIA	Required						<i>Trogoderma granarium</i>	<i>Bidens pilosa</i> , <i>Solanum triflorum</i> , <i>Solanum elaeagnifolium</i> , <i>Cenchrus pauciflorus</i> , <i>Ipomoea hederacea</i> , <i>Ipomoea lacunosa</i> , <i>Solanum carolinense</i> , <i>Ambrosia atermisifolia</i> , <i>Ambrosia psilostachya</i> , <i>Ambrosia trifida</i> , <i>Cuscuta</i> spp., <i>Solanum rostratum</i>		Required		
SAUDI ARABIA	Required											
SERBIA	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica</i>							
SOMALIA										Required		
SOUTH AFRICA	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Alternaria triticina</i> , <i>Tilletia controversa</i> , <i>Tilletia indica</i> , <i>Urocystis agropyri</i>	<i>Anguina tritici</i>				Required		
SPAIN	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.								Required		
SWEDEN	Required											
SWITZERLAND		The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.			<i>Tilletia indica</i>							
TAJIKISTAN	Required	Must be free of all pests and weeds not present in Tajikistan.										Airtight packaging required.
TANZANIA	Required				<i>Tilletia indica</i>	<i>Anguina tritici</i>						
TUNISIA			Barley Stripe Mosaic Virus	<i>Xanthomonas translucens</i> pv. <i>translucens</i>		<i>Anguina tritici</i>						
TURKEY	Required	Non-commercial invoice and list of germplasm required.  Seed samples are free from all types of diseases and not transgenic. Statement as indicated required re <i>Tilletia indica</i> (either not found in area grown or not found in observation/testing).			<i>Tilletia indica</i>				Required (to be sent with shipment)	Required		
UKRAINE	Required											
UNITED ARAB EMIRATES	Required											

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
URUGUAY	Required	Must be accompanied by International Phytosanitary Certificate specifying the import permit number.  The seed multiplication plot was officially inspected at least once during growth and found free of [named pathogens] OR The sample is free of [named pathogens] according to official laboratory analysis [laboratory specified].	Barley Stripe Mosaic Virus		<i>Tilletia indica</i>		<i>Trogoderma variabile</i>	<i>Cirsium arvense</i> , <i>Lepidium draba</i> , <i>Lolium rigidum</i> , <i>Thlaspi arvense</i>			Insecticide treatment suitable to eliminate <i>Trogoderma variabile</i> (product, concentration, temperature and time must be specified).	
UNITED STATES of AMERICA	Required	Must be free from soil, weeds, and pests.  All costs and arrangements for transportation to and from the PGIS, is the responsibility of the importer.			<i>Tilletia indica</i> , <i>Urocystis agropyri</i>			Free of weed seeds including noxious weeds.			Required	
UZBEKISTAN	Required	The seed was produced in a Karnal bunt ( <i>Tilletia indica</i> ) free area.					<i>Trogoderma granarium</i>	<i>Ambrosia trifida</i> , <i>Ambrosia psilostachya</i> , <i>Solanum rostratum</i> , <i>Solanum triflorum</i> , <i>Acroptilon repens</i> , <i>Cenchrus pauciflorus</i>				
ZAMBIA	Required		Barley Yellow Dwarf Virus, Brome Mosaic Virus	<i>Acidovorax avenae</i> subsp. <i>avenae</i>	<i>Gaeumannomyces graminis tritici</i> <i>Tilletia controversa</i> , <i>Tilletia indica</i> , <i>Tilletia tritici</i> , <i>Tilletia laevis</i> , <i>Typhula idahoensis</i> , <i>Urocystis agropyri</i> , <i>Ustilago hordei</i>			<i>Ambrosia artemisifolia</i> , <i>Anagallis arvensis</i> , <i>Cirsium arvense</i> , <i>Cardaria draba</i> , <i>Fumaria officinalis</i> , <i>Lolium temulentum</i> , <i>Papaver rhoeas</i> , <i>Phalaris minor</i> , <i>Melilotus indica</i> , <i>Thlaspi arvense</i>		Required		
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, Barley Yellow Dwarf Virus, Brome Mosaic Virus	<i>Clavibacter iranicus</i> , <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> , <i>Pseudomonas syringae</i> , <i>Rathayibacter iranicus</i> , <i>Xanthomonas translucens</i>	<i>Alternaria triticina</i> , <i>Claviceps purpurea</i> , <i>Gibberella zeae</i> , <i>Monographella nivalis</i> , <i>Pyrenophora seminiperda</i> , <i>Pyrenophora tritici-repentis</i> , <i>Sclerophthora macrospora</i> , <i>Tilletia</i> spp., <i>Urocystis agropyri</i> , <i>Ustilago nuda</i> f.sp. <i>tritici</i>	<i>Anguina tritici</i>				Required	Treatment with appropriate fungicide and insecticide or fumigant.	

**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 requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
ALGERIA	Required									required		
ANGOLA (Rep. of)	Required											
ARGENTINA	Required			<i>Pantoea stewartii</i>			<i>Trogoderma versicolor</i> , <i>Trogoderma variabile</i> , <i>Diabrotica virgifera virgifera</i>					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.
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				<i>Trogoderma</i> 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 be presented to AQIS at the time of clearance. Shipments must to be addressed to AQIS as stated in the permit.
BANGLADESH	Required			<i>Pantoea stewartii</i>	<i>Peronosclerospora philippensis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora maydis</i> <i>Sclerophthora rayssiae</i> var. <i>zeae</i>							
BELIZE	Required	Seed must be free of live insects, soil, and diseases of quarantine concern.  Seed must be free from weeds.								Required		
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).					<i>Prostephanus truncatus</i> , <i>Trogoderma variabile</i>			Required		Must arrive in new containers and cardboard or wood packaging, packed in a clean and sanitised way that permits phytosanitary inspection.
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.		<i>Pantoea stewartii</i> , <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>	<i>Acremonium maydis</i> , <i>Kabatiella zeae</i> , <i>Peronosclerospora maydis</i> , <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora sorghi</i> , <i>Sclerophthora rayssiae</i> var. <i>zeae</i> , <i>Cercospora sorghi</i> , <i>Mycosphaerella zeae-maydis</i>	<i>Heterodera zeae</i>	<i>Trogoderma granarium</i> , <i>Prostephanus truncatus</i>	<i>Striga</i> 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.
CAMBODIA	Required											
CANADA	Required								Required			
CHILE	Required										Carboxin+Captan or Carboxin+Thiram	
CHINA	Required	Shipping requirements depend on province. Any requirements are listed on the permit.	Maize Chlorotic Mottle Virus		<i>Peronosclerospora sorghi</i>		<i>Prostephanus truncatus</i> , <i>Diabrotica virgifera</i>	<i>Tribolium dstructor</i> , <i>Ambrosia</i> spp.				

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
COLOMBIA	Required	Seed for experimental use/Semilla para uso experimental. Commercial value 3USD.		<i>Pectobacterium chrysanthemi</i>	<i>Peronosclerospora maydis</i> , <i>Peronosclerospora sorghi</i> , <i>Peronosclerospora philippinensis</i> , <i>Sclerophthora macrospora</i> , <i>Sclerophthora rayssiae</i> var. <i>zeae</i> , <i>Cercospora zea-maydis</i> , <i>Mycosphaerella</i> spp.		<i>Diabrotica virgifera virgifera</i> , <i>Trogoderma granarium</i>		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.
COSTA RICA	Required											
CUBA	Required		Sugarcane Mosaic Virus, Maize Dwarf Mosaic Virus	<i>Pantoea stewartii</i> , <i>Pseudomonas syringae</i> , <i>Burkholderia andropogonis</i>	<i>Sclerophthora macrospora</i>	<i>Ditylenchus dipsaci</i>	<i>Tenebrio molitor</i> , <i>Trogoderma granarium</i>	<i>Agropyron repens</i> , <i>Amaranthus retroflexus</i> , <i>Arceuthobium</i> spp., <i>Convolvulus arvensis</i> , <i>Cuscuta</i> spp., <i>Digitaria velutina</i> , <i>Orobancha</i> spp., <i>Pennisetum macrourum</i> , <i>Solanum rostratum</i> , <i>Sonchus arvensis</i>				
ECUADOR	Required			<i>Acidovorax avenae</i> subsp. <i>avenae</i> , <i>Clavibacter michiganensis nebraskensis</i> , <i>Erwinia chrysanthemi</i> , <i>Pantoea stewartii</i> , <i>Xanthomonas campestris</i> pv. <i>vasculorum</i>	<i>Claviceps gigantea</i> , <i>Gibberella avenacea</i> , <i>Gibberella moniliformis</i> , <i>Kabatella zeae</i> , <i>Micospherella pinodes</i> , <i>Micospherella zea-maydis</i> , <i>Pseudoperonospora sorghi</i> , <i>Sarocladium oryzae</i>		<i>Atherigona oryzae</i> , <i>Busseola fusca</i> , <i>Caulophilus oryzae</i> , <i>Cryptolestes pusillus</i> , <i>Graphognathus leucoloma</i> , <i>Ostrinia nubilalis</i> , <i>Prostephanus truncatus</i> , <i>Rhyzopertha dominica</i> , <i>Sesamia cretica</i> , <i>Sitophilus zeamays</i> , <i>Spahcelotheca cruenta</i> , <i>Spahcelotheca gigantes</i> , <i>Trogoderma</i> spp.				Fumigation with aluminum phosphide for 72 hours at 3-4 tablets per ton and treatment with a protective fungicide	The seed must be sent in new containers.
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.								Required		
EL SALVADOR	Required									Required		
ETHIOPIA (Federal Democratic Rep. of)	Required	Free from soil The crop must have been inspected during active growth.		<i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Peronosclerospora sacchari</i>		<i>Prostephanus truncatus</i>			Required	fungicide and insecticide always required and must be stated on the International Phytosanitary Certificate	
FIJI	Required		Maize Dwarf Mosaic Virus		<i>Ustilago maydis</i>					Required	fungicide and insecticide required	
FRANCE	Required											
GEORGIA	Required			<i>Pantoea stewartii</i>	<i>Cochliobolus heterostrophus</i> , <i>Stenocarpella macrospora</i>		<i>Diabrotica virgifera virgifera</i> , <i>Trogoderma granarium</i>					
GERMANY	Required	Seeds for scientific purposes, or for the purpose of breeding and exhibition.								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).
GHANA	Required	Certificate of Origin and Commercial Invoice required.			<i>Claviceps gigantea</i>		<i>Trogoderma variabile</i> , <i>Carpophilus dimidiatus</i>	<i>Cirsium arvense</i>		Required		
GUATEMALA	Required											
HAITI	Required											

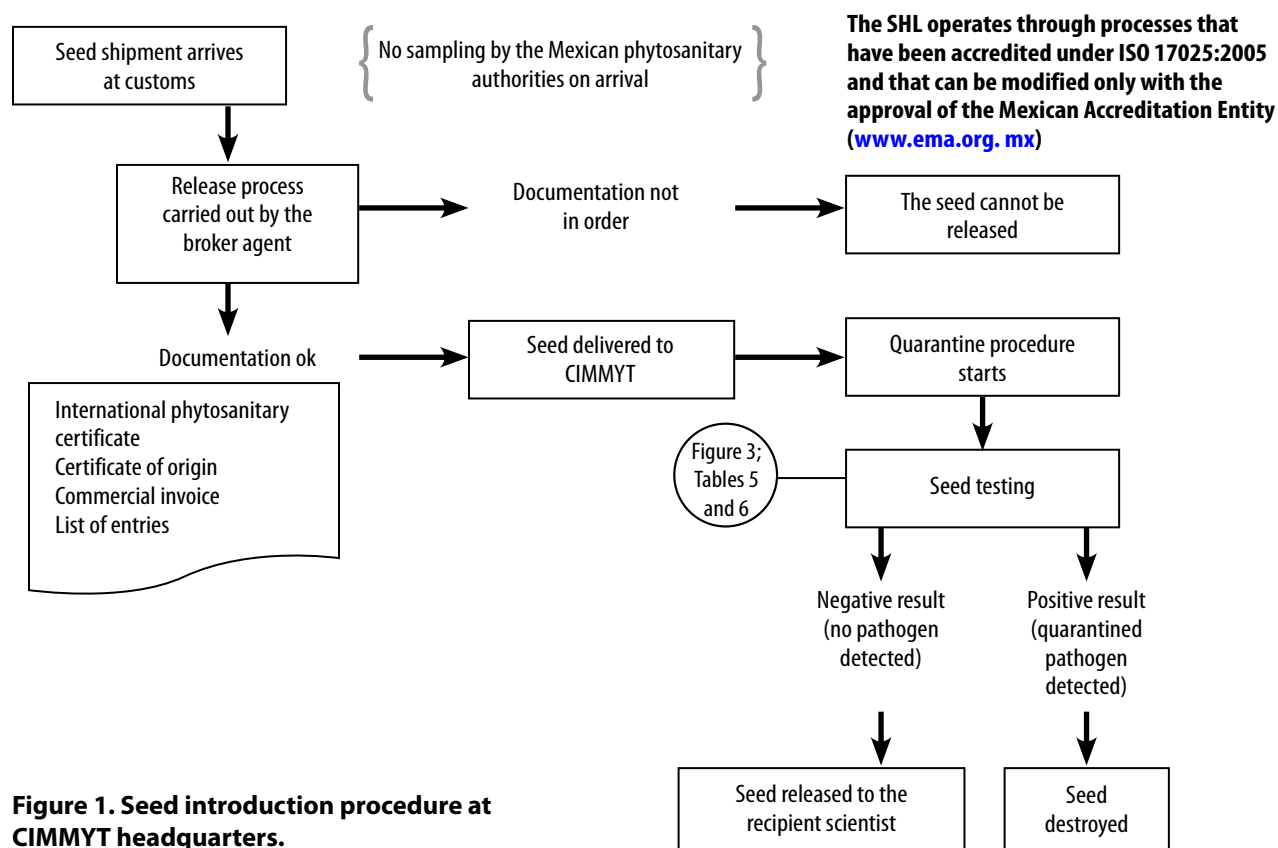
			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
INDIA	Required	The seed must be free from soil.	Maize Chlorotic Mottle Virus Wheat Streak Mosaic Virus	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> , <i>Pantoea stewartii</i> , <i>Burkholderia andropogonis</i> , <i>Pantoea agglomerans</i> , <i>Pseudomonas fuscovaginae</i> , <i>Pseudomonas syringae</i> pv. <i>coronafaciens</i>	<i>Claviceps gigantea</i> , <i>Cochliobolus heterostrophus</i> , <i>Phakopsora zeae</i> , <i>Kabatiella zeae</i> , <i>Mycosphaerella zeae-maydis</i>		<i>Prostephanus truncatus</i> , <i>Sitophilus zeamays</i>		Required, to be sent at the same time as the shipment			
INDONESIA	Required											
IRAN	Required		High Plain Virus, Maize Chlorotic Mottle Virus	<i>Pantoea stewartii</i>	<i>Glomerella graminicola</i> , <i>Stenocarpella maydis</i> , <i>Claviceps gigantea</i> , <i>Stenocarpella macrospora</i>		<i>Araecerus fasciculatus</i>					
ITALY	Required									Required		
JAMAICA	Required	Package and contents to be free from soil. All plant material to be accompanied by an official 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.				<i>Globodera rostochiensis</i>						Packing material to consist of moss, paper, wood shavings or other artificial media.
JAPAN	Required			<i>Pantoea stewartii</i>								
KENYA	Required	Plants or plant parts must be entirely free from soil, chaff, and/or leaf mould. Seed must be treated.  Plants were field inspected during active growth and found to be free from <i>Sclerospora graminicola</i> (Sacc) Schroet and S. sacchari. <i>Xanthomonas stewartii</i> (E F Smith) Dowson is not known to occur in the area of production/The seeds were tested and found to be free from <i>Xanthomonas stewartii</i> (E F Smith) Dowson.		<i>Pantoea stewartii</i>	<i>Sclerospora graminicola</i> , <i>Sphacelotheca reiliana</i> , <i>Peronosclerospora sacchari</i> , <i>Ustilago maydis</i>					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 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).
KYRGYZSTAN	Required											
MADAGASCAR	Required		Maize Dwarf Mosaic Virus	<i>Pantoea stewartii</i> , <i>Pseudomonas syringae</i>	<i>Peronosclerospora sorghi</i> , <i>Sclerophthora macrospora</i> , <i>Scelrospora graminicola</i>	<i>Ditylenchus dipsacii</i>						
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-Virus									
MALAWI	Required		Free from virus diseases	<i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Cochliobolus heterostrophus</i> , <i>Kabatiella zeae</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora spontanea</i>		<i>Trogoderma granarium</i> , <i>Prostephanus truncatus</i>			Required	Required	The material does not contain any cytoplasmic male sterile lines.

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
MAURITIUS	Required	Certified free from dangerous diseases and pests.	Maize Dwarf Mosaic Virus, Sugarcane Mosaic Virus, Wheat Streak Mosaic Virus	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> , <i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Claviceps gigantea</i> , <i>Cochliobolus heterostrophus</i> , <i>Helminthosporium maydis</i> race T, <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora sorghi</i>		<i>Prostephanus truncatus</i>				Required (insecticide and fungicide, with description)	Must not be packed in earth, leaf or garden mould, compost, farmyard manure, straw or unsterilized sphagnum moss.
MEXICO	Required		Wheat Streak Mosaic Virus	<i>Burkholderia andropogonis</i> , <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> , <i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Peronosclerospora sacchari</i> , <i>Sclerophthora rayssiae</i> var. <i>zeae</i> <i>Peronosclerospora philippinensis</i>	<i>Heterodera zeae</i>	<i>Busseola fusca</i> , <i>Ostrinia nubilalis</i>					
MOZAMBIQUE	Required		Maize Dwarf Mosaic Virus	<i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Claviceps gigantea</i> , <i>Kabatiella zeae</i> , <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora sacchari</i> , <i>Sclerophthora rayssiae</i> var. <i>zeae</i>		<i>Prostephanus truncatus</i>					
NEPAL	Required											
The NETHERLANDS	Required	Inspection at entrance.										
NICARAGUA	Required	Must be accompanied by Certificate of Origin.	Maize Dwarf Mosaic Virus	<i>Pantoea stewartii</i>	<i>Claviceps gigantea</i>	<i>Ditylenchus dipsaci</i>	<i>Caulophilus oryzae</i>			Required		
NIGERIA	Required		Maize Dwarf, Maize Rough Virus	<i>Clavibacter michiganensis nebraskensis</i> , <i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Peronosclerospora maydis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora spontanea</i> , <i>Puccinia polysora</i> , <i>Sclerophthora macrospora</i> , <i>Ustilago maydis</i>		<i>Diabotrica virgifera virgifera</i> , <i>Prostephanus truncatus</i> , <i>Trogoderma inlcusum</i> , <i>Listronotus bonanriensis</i>			Required		
PAKISTAN	Required	Must be free from soil and weeds certified free from insect pests and diseases. Seed consignment must be accompanied with seed testing report issued from Government Laboratory of country of origin showing details of germination, abnormal seeds, inert materials, weed seeds, year of production and seed pathology.		<i>Pantoea stewartii</i>	<i>Pantoea stewartii</i>	<i>Peronosclerospora</i> spp.				Required	Fungicide treatment required (name and dose should be stated on IPC)	
PANAMA	Required											
PAPUA NEW GUINEA	Required											
PARAGUAY	Required											
PERU	Required			<i>Pantoea stewartii</i> , <i>Pseudomonas syringae</i> pv. <i>syringae</i>	<i>Cochliobolus heterotrophus</i> , <i>Stenocarpella maydis</i> , <i>Stenocarpella macrospora</i> , <i>Sphaceloteca reiliana</i>		<i>Trogoderma variabile</i> , <i>Trogoderma versicolor</i>		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 confirmation.
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.		<i>Pseudomonas syringae</i> pv. <i>syringae</i>	<i>Claviceps gigantea</i> , <i>Cercospora zeae-maydis</i>		<i>Prostephanus truncatus</i> , <i>Trogoderma variabile</i>			Required	Fungicide and insecticide treatment required (to be stated in IPC)	Seeds should be properly packed.
SERBIA	Required			<i>Pantoea stewartii</i>						Required		
SOUTH AFRICA (Rep. of )	Required			<i>Pantoea stewartii</i>	<i>Acremonium maydis</i> , <i>Cochliobolus heterostrophus</i> , <i>Kabatiella zeae</i> , <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora maydis</i> , <i>Sclerophthora rayssiae</i> var. <i>zeae</i> ,		<i>Prostephanus truncatus</i> , <i>Trogoderma granarium</i> , <i>Listronotus bonariensis</i> , <i>Chaetocnema pulicari</i> , <i>Diabotrica virgifera</i>			Required		

			Phytosanitary requirements, seed free from:									
Country	Import permit	Additional requirements	Pathogens			Nematodes	Insects	Weeds	Untreated sample	Non GMO declaration	Chemical treatment requirements	Shipping requirements
			Virus	Bacteria	Fungi							
SPAIN	Required											
TAJIKISTAN	Required											
TANZANIA	Required			<i>Pantoea stewartii</i>	<i>Peronosclerospora sorghi</i> , <i>Peronosclerospora saccharii</i>						Required	
THAILAND	Required									Required		
TUNISIA	Required				<i>Acremonium maydis</i> , <i>Cochliobolus carbonum</i> , <i>Diplodia</i> spp., <i>Gibberella moniliformis</i> , <i>Gibberella zeae</i>							
TURKEY	Required											
UGANDA	Required			<i>Pantoea stewartii</i>	<i>Cochliobolus heterostrophus</i> <i>Peronosclerospora</i> spp.					Required		
UNITED STATES of AMERICA	Required	The shipment must be free from soil, plant material other than seed, other foreign matter or debris, seeds in the fruit or seed pod, and living organisms such as parasitic plants, pathogens, insects, snails, and mites. The seeds must be free from pesticides.										<p>The shipment should be sent to the Plant Inspection Station (PIS) at the port(s) specified on the permit using supplied ""green and yellow"" permit label(s), with no other delivery address on the outside of the package.</p> <p>The seeds must be securely packaged in packets or envelopes and sealed to prevent spillage. Clear plastic re-sealable bags are highly recommended. Regulations apply to wood packaging.</p> <p>A typed or legibly printed seed list/invoice must accompany each shipment with the name of the collector/shipper, the botanical names (at least to genus, preferably to species level) listed alphabetically, and the country of origin and country shipped from for each taxon. Each seed packet must be clearly labeled with the name of the collector/shipper, the country of origin, and the scientific name. Or, the seed list may provide a code for each lot, which may be used on the seed packets. In this case, each packet must at least include the code and scientific name used.</p> <p>Each packet must be limited to a maximum of 50 seeds or 10 grams of seed of 1 taxon (genus, species, cultivar, etc.) per packet. Each shipment must contain no more than 50 seed packets.</p>
URUGUAY	Required			<i>Pantoea stewartii</i>		<i>Aphelenchoides besseyi</i>		<i>Cirsium arvense</i>				
VIETNAM	Required											
YEMEN	Required											
ZAMBIA	Required	The seed must be treated.	Sugarcane Mosaic Virus, Barley Yellow Dwarf Viruses	<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> , <i>Pantoea stewartii</i> , <i>Pseudomonas syringae</i> pv. <i>coronafaciens</i> , <i>Acidovorax avenae</i> subsp. <i>avenae</i>	<i>Claviceps gigantea</i> , <i>Peronosclerospora philippensis</i> , <i>Peronosclerospora sacchari</i> , <i>Sclerophthora macrospora</i>			<i>Cirsium arvense</i> , <i>Raphanus raphanistrum</i>		Required	Wide spectrum pesticide	
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.	Maize Leaf Spot Virus, Sugarcane Mosaic Virus	<i>Pantoea stewartii</i> , <i>Pseudomonas syringae</i> , <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>	<i>Acremonium strictum</i> , <i>Claviceps gigantea</i> , <i>Helminthosporium maydis</i> race T, <i>Lasiodiplodia theobromae</i> , <i>Peronosclerospora maydis</i> , <i>Peronosclerospora philippinensis</i> , <i>Peronosclerospora sacchari</i> , <i>Peronosclerospora sorghi</i> , <i>Sclerophthora macrospora</i> , <i>Stenocarpella maydis</i> , <i>Ustilago maydis</i>		<i>Prostephanus truncatus</i> , <i>Rhyzopertha dominica</i>			Required	Treatment with fungicides required	



## Seed health procedures for incoming seed



**Figure 1. Seed introduction procedure at CIMMYT headquarters.**

### 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 permit 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 that has no expiration date prior to receipt in Mexico of any maize, wheat, or triticale seed. In 2012 CIMMYT and the Mexico's plant health authority, *Dirección General de Sanidad Vegetal* (DGSV), 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 permanent seed import authorization issued by Mexico normally takes 3-5 days to be granted, and remains valid until 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 required original document is missing, the entire shipment will be 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 under separate cover 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 of 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, bulbs, etc.)
- Fungal and bacterial cultures or isolates
- DNA and RNA extracts
- Plasmids
- Soil

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, 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 two signatories approved by the Government Phytosanitary Office (DGSV) and that every two years must renew their approbation are the official authorities for making diagnostic and sign the documents that allow the release of the seed.

The SHL’s official status facilitates seed importation into Mexico (and CIMMYT) 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 internal 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. 45).

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](#). 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.**

**Introduction blocks**

Seed meeting the requirements described above may be released and planted without exception in the introduction blocks. These introduction blocks, are dedicated plots on CIMMYT's Mexican research stations set aside for the planting of newly-introduced maize or wheat seed. The purpose of this procedure is to monitor, detect, and destroy any pathogen that may have not been detected by laboratory tests.

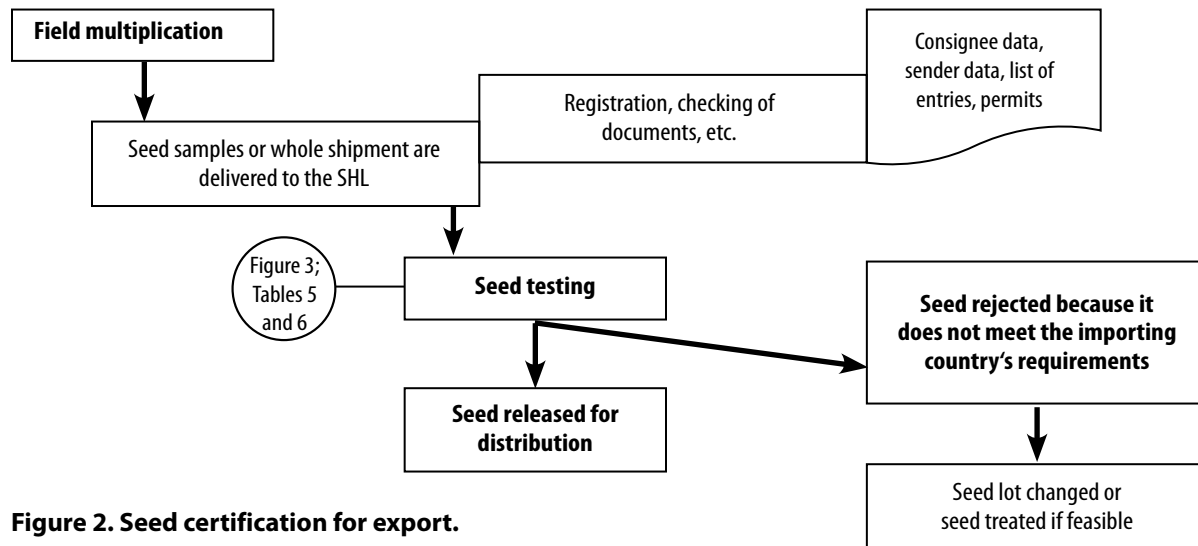
All introduction blocks are grown in complete isolation, and intercropping of other materials is not allowed. Introduced seed will be monitored during the growth cycle by SHL staff and the DGSV inspector.

Each program will provide lists of all materials to be grown in quarantine plots before each cycle and in every location.

To facilitate inspection, materials being grown on introduction blocks should be labeled as soon as possible. The SHL introduction number, number of entries, and country of origin must be included on each tag.

All decisions regarding chemical spraying will be taken jointly by program scientists and SHL staff. Plants in the introduction blocks that show symptoms of unusual diseases will be removed, samples will be taken to the SHL for examination, and the remaining tissue will be destroyed.

## Seed health procedures for outgoing seed



**Figure 2. Seed certification for export.**

### 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. 45). Additional tests are conducted upon request if the country of destination has special requirements.

## Wheat

### Seed multiplication in Karnal bunt-free areas

The Mexicali area, in northwestern Mexico has been officially declared free of Karnal bunt (KB) according to the *Diario Oficial de la Federación* published on 15 December 1997, and CIMMYT El Batán, State of Mexico, experiment station has been declared Karnal bunt free published on the *Diario Oficial de la Federación* published on October 20, 2009 following instructions given in *Norma Oficial Mexicana* NOM-001-FITO-2001. These areas are also recognized as KB-free by the North American Plant Protection Organization (NAPPO) and the European Plant Protection Organization (EPPO).

The CIMMYT Wheat Program uses fields in Mexicali for multiplying seed intended for export.

Wheat and triticale germplasm to be included in an international nursery must be planted in plots assigned for that purpose at CIMMYT's headquarters at El Batán.

During the crop cycle, 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 aerial infection by *T. indica*.

### Washing procedure

After harvest, the seed is washed with a 1.2% sodium hypochlorite solution to destroy any possible teliospore 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 (Vitavax 300, 3 g/kg of seed) and chlorothalonil (Daconil 2787, 2 g/kg of seed). The seed is then shipped to Mexicali to be sown and multiplied. (see "Washing procedure," p. 42)

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 following the method described above. 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 inspected at the end of the cycle, close to harvest time. Randomly sampled spikes are collected, individually threshed, and examined to detect potential seed health problems. The seed then undergoes a filter wash test. If the test results are negative, each line is individually harvested.

The harvested seed is placed 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 appropriately packed and 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 1.2% 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 non-contamination.

Lines received in the international nurseries area can now undergo final selection by the program head for inclusion in international trials. The selected lines are cleaned by sifting 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/inch<sup>2</sup>);
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 filter test for the presence of *T. indica* and other *Tilletia* spp. teliospores.

While the tests are being conducted, the remaining seed, after washing, is treated with a slurry containing chlorothalonil (Daconil 2787, 2 g/kg of seed) and carboxin + captan (Vitavax 300, 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 *T. indica* teliospores, or any other 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 (see “Miscellaneous shipments” below) must follow this same procedure.

### **Miscellaneous shipments**

“Miscellaneous” shipments contain seed that has not been through a multiplication cycle in Mexicali, but which is required to be sent to collaborators. 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 Karnal bunt and other diseases of quarantine concern. SHL staff decide whether to test every line or make up a composite sample for testing. Seed must be washed and chemically treated (as described above) before shipment.

Seed produced on CIMMYT’s Yaqui Valley, Sonora research station must go through a multiplication cycle in El Batán before export or multiplication in Mexicali.

### **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 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 Maize Program to the SHL falls into three groups: international nurseries, miscellaneous nurseries, and CIMMYT maize lines. 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: thiodicarb (Semevin 350 SA), metalaxil-M (ApronXL), captan, thiophanate-methyl (Maxim XL), adherent.

# **Rules 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 fungicide-treated seed unless absolutely necessary.
- Seed is rechecked for KB before shipping to collaborators.

Seed is stored in metal 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 1.2% 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 *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. 42.
- All seed must be treated with chlorothalonil (Daconil 2787, 2 g/kg of seed).
- 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:

- Do not take seed or other plant parts or soil from the field or storehouses. Please contact the station superintendent (Ing. Rodrigo Rascon) or the scientist in charge of the program for instructions and procedures to follow.
- Wash your clothes and shoes if you've been in the field, before going back home.
- Wash (inside and out) vehicles used in the field, before travelling to KB-free areas.
- Wash or disinfect with a 1.2% chlorine water solution or alcohol all equipment or instruments used in the field, before returning them.



## 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 generally uses well-established test procedures that may be found in any standard reference on seed health (see References). The filter wash test is somewhat specialized (see p. 48). 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 and takes two weeks.
- **Greenhouse germination test**, for the expression, and thus detection, of seed-borne 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.

Details of the tests and their target pathogens are summarized in Tables 1 (maize) and 2 (wheat and triticale); a flowchart of the test procedures is given in Figure 3.

**Table 5. Seed health testing on maize.**

Test	Pathogen type(s) detected	Pathogens of importance in incoming seed	Pathogens of quarantine importance in outgoing seed **
Seed wash filter test	Fungi (smuts)		<i>Ustilago maydis</i>
	Nematodes	<i>Heterodera zeae</i> *	
Freezing blotter test	Imperfect fungi	<i>Acremonium maydis</i> *	<i>Cochliobolus</i> spp. <i>Dilpodia</i> spp. <i>Fusarium</i> spp. <i>Lasiodiplodia theobromae</i>
Greenhouse germination test	Bacteria	<i>Burkholderia andropogonis</i> * <i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i> * <i>Pantoea stewartii</i> *	<i>Acidovorax avenae</i> subsp. <i>avenae</i> <i>C. michiganensis</i> subsp. <i>nebraskensis</i> <i>P. stewartii</i>
	Viruses	Wheat Streak Mosaic Virus*	Maize Dwarf Mosaic Virus Maize Chlorotic Dwarf Virus Sugarcane Mosaic Virus
ELISA	Bacteria	<i>Pantoea stewartii</i> *	<i>P. stewartii</i>
	Viruses	Wheat streak mosaic virus*	Maize dwarf mosaic virus Maize chlorotic dwarf virus Sugarcane mosaic virus
Downy mildew detection test		<i>Peronosclerospora maydis</i> * <i>P. philippinensis</i> * <i>P. sacchari</i> * <i>P. sorghi</i> <i>Sclerophthora rayssiae</i> var. <i>zeae</i> *	<i>P. sorghi</i>
PCR		<i>Pantoea stewartii</i>	<i>Pantoea stewartii</i>

\* Quarantined under *Norma Oficial Mexicana* NOM-018-FITO-1995.

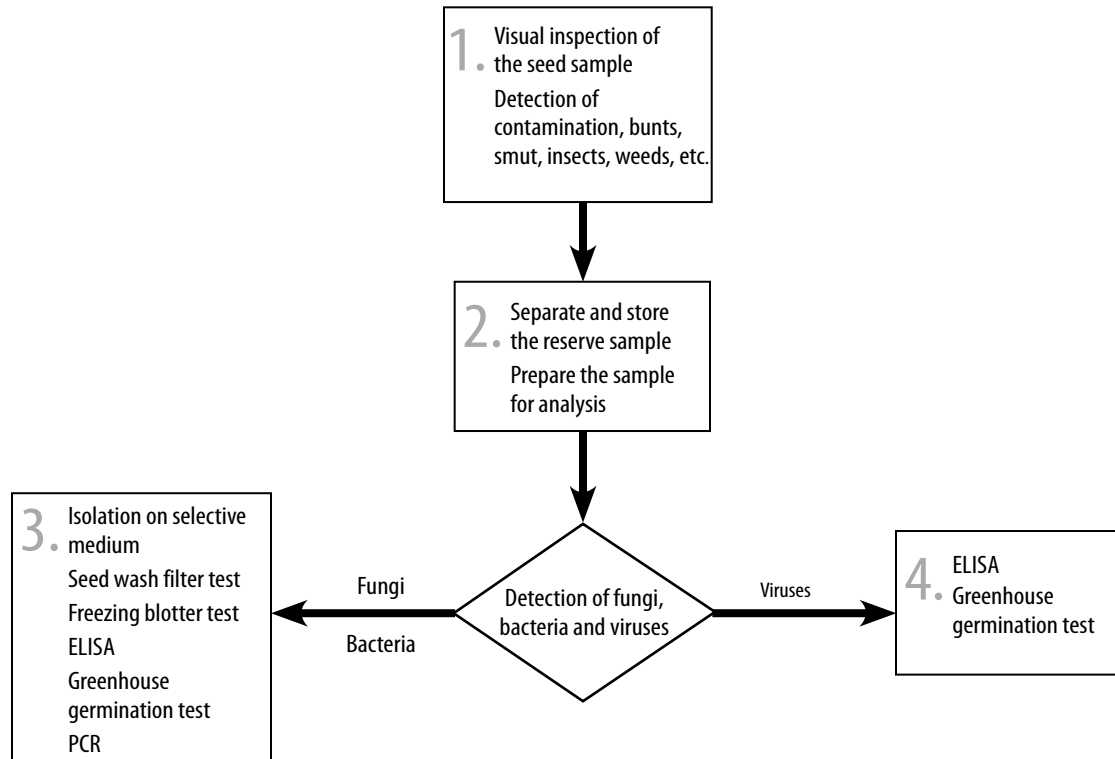
\*\* According to information reported on importing countries requirements

**Table 6. Seed health testing on wheat and triticale.**

Test	Pathogen type(s) detected	Pathogens of importance in incoming seed	Pathogens of quarantine importance in outgoing seed **
Seed wash filter test	Fungi: bunts	<i>Tilletia indica</i> *, <i>Tilletia controversa</i> *	<i>Tilletia indica</i> <i>Tilletia</i> spp
	smuts		<i>Ustilago</i> spp
	Nematodes	<i>Anguina tritici</i> *	.
Freezing blotter test	Imperfect fungi	<i>Alternaria tritricina</i> *	<i>Fusarium</i> spp. <i>Helminthosporium</i> spp. <i>Septoria</i> spp.
Greenhouse germination test	Bacteria	<i>Pseudomonas syringae</i> pv. <i>atrofaciens</i> * <i>Xanthomonas translucens</i> pv. <i>undulosa</i>	<i>P. syringae</i>
	Viruses	Barley Stripe Mosaic Virus Wheat Streak Mosaic Virus	
ELISA	Viruses	Barley Stripe Mosaic Virus Wheat Streak Mosaic Virus	Barley Stripe Mosaic Virus Wheat Streak Mosaic Virus
PCR		Bacteria	Bacteria

\* Quarantined under *Norma Oficial Mexicana* NOM-017-FITO-1995.

\*\* According to information reported on importing countries requirements



**Figure 3. Pathogen detection and identification flowchart.**

## References

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- ISTA. 2002. *Handbook on Seed Health Testing*. Section 2: Working Sheets. Edited by J. Jorgensen. Zürich: International Seed Testing Association.
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- McGee, D.C. 1988. *Maize Diseases: A Reference Source for Seed Technologists*. St. Paul, MN: APS Press. 150 pp.
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## 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  $\mu$ m polyester or nylon meshes. Large spores and debris will remain on the 50  $\mu$ m mesh, and *Tilletia indica* teliospores, with an average diameter of 25-40  $\mu$ m, will be

caught on the 15  $\mu$ 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  $\mu$ 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  $\mu$ m. Similarly, after filtering, it should be moistened with 3% KOH and observed under a stereo microscope.

# Templates

## Letter of commercial value template

[Letterhead of Issuing Institution/Company]  
[En papel membretado de la institución o empresa]

Date (Fecha): \_\_\_\_\_

Commercial Letter  
Carta Comercial

To whom it may concern:  
A quien corresponda:

Box(es) containing \_\_\_\_\_ kg of (indicate which species) seed samples donated for research purposes, with no commercial value and an estimated value "for customs purposes only" of: \_\_\_\_\_\*

Caja(s) que contienen \_\_\_\_\_ kg de muestras de semilla de (indicar cual especie) donadas para uso experimental sin valor comercial, con un valor aproximado "solo para propositos aduanales" de: \_\_\_\_\_\*

Shipper's Signature  
Firma del Consignatario

\_\_\_\_\_

- \* Amount suggested is US \$0.50 per kg of seed, but do not declare a total amount lower than US \$1.00 or higher than US \$100.00.
- \* 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) \_\_\_\_\_

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