

Pest Risk Analysis (PRA) and Quarantine Precautions for Maize Importation into Ethiopia: A Case of Eleven Source Countries

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ABSTRACT

Maize improvement program in Ethiopia utilizes a large amount of germplasm from external sources. Seed samples were imported from eleven different countries Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt from 2011 to 2020. During maize importation, regular inspection was carried out at Holetta Agricultural Research Center for freedom from pests of insects, pathogens and weed seeds. In order to be effective in this important regulatory pest management undertaking, this review attempts to provide a concerted approach for effecting pre- and post-entry regulatory measures for maize importation into Ethiopia based on pest risk analysis (PRA) to safeguard the country. A total of 18 pests (including 12 arthropods, 3 fungi, 1 bacterium, 1 virus and 1 *Spiroplasma*) are of quarantine concern when importing maize seed into Ethiopia from the eleven major germplasm source-countries considered in this PRA. Three fungi (*Cochliobolus*, *Fusarium* and *Mycosphaerella*), one bacterium bacterial wilt of maize (*Pantoea stewartii*) and the virus maize chlorotic dwarf virus and corn stunt *Spiroplasma* (*Spiroplasma kunkelii*) and also twelve arthropods are of quarantine concern to the country. The number and species of pests of quarantine concern for the country vary depending on country of origin the highest in Mexico with 11 pests followed by India (7 pests) and then Colombia (6 pests). The rest consists of only 1–5 pests of quarantine importance for Ethiopia. Therefore, this review describes quarantine precautions in the import control scenarios and presents PRA based on pathway analysis for importing maize seed into Ethiopia from eleven major source-countries. Generally, the importers of maize into Ethiopia need to strictly consider the information provided in this paper before, during and after arrival of maize samples for research.

Keywords: Germplasms; Maize; Pathways; Pest Risk Analysis (PRA); Quarantine precautions

INTRODUCTION

The maize improvement program in Ethiopia utilizes a large amount of germplasm from external sources, and thus, over 34,000 maize seed samples were imported from different countries including Mexico, Kenya, Zimbabwe, Nigeria and Syria from 2001 to 2008 [1]. And also thus, over 4,6268 maize seed samples were imported from different countries including Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt from 2011 to 2020 [2]. Presently, Ethiopia imports an average of 4,627 maize seed samples every year [2]. Uganda, USA, Syria and Burkina Faso were also among major sources in the past [3,4].

Quarantine precautions against inadvertent importation of pests during germplasm and technology exchange have been an important topic in the world for many years. Accordingly, periodic reviews

of quarantine information were published on maize importation into Ethiopia [3,4] to increase awareness in the country. Plant quarantine guidelines for the national agricultural research system (NARS) were published by the Ethiopian Institute of Agricultural Research (EIAR) [5] to support the risk management operations at the national level. During maize importation, regular inspection was carried out at Holetta Agricultural Research Center (HARC) of the EIAR for freedom from pests that include insects, pathogens and weed seeds. Reports indicated that until 1990 alone 45 maize shipments were destroyed by appropriate methods due to infestation with serious and risky pests [6]. Additionally, in accordance with our inspection guidelines and procedures, about 12.6% of the maize materials imported into the country were cleaned, sorted or treated with appropriate seed treatment to safeguard the country from alien pests of quarantine concern.

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METHODOLOGY

Inspection procedures followed in the past were those recommended in previous reviews [3,4] and more recently, additional post-entry follow-ups were carried out in accordance to Dereje [5]. At present, however, importation of plant materials into a country necessitates a consideration of recent advances in regulatory sciences for sound biological, economic, social and policy decisions. Current trends in quarantine inspection and post-entry follow-ups for any plant commodity require guidelines and procedures outlined by the sanitary and phytosanitary standards (SPS) that conforms to the terms of the International Plant Protection Convention, rectified by Ethiopia [1]. In order to be effective in this important regulatory pest management undertaking, this review attempts to provide a concerted approach for effecting pre- and post-entry regulatory measures for maize importation into Ethiopia based on pest risk analysis (PRA). Accordingly, this paper describes quarantine precautions in the import control scenarios and presents PRA based on pathway analysis for importing maize seed into Ethiopia from eleven major source-countries including Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt. It also provides protocols for inspection and detection, and phytosanitary measures for potentially risky pests to safeguard the country. Finally, it proposes a list of important considerations for the future.

Mechanism of import control

The mechanism of plant quarantine operates under five sets of guiding principles and procedures comprising embargoes, inspection and certification, disinfection, special permits, and unrestricted shipments. In order to be effective, both pre- and post-entry quarantine measures are very important and complementary. Pre-entry quarantine includes importing maize from pest-free areas, field inspection at the country of origin, and laboratory tests and seed treatment at the country of origin based on the results of PRA. Post-entry follow-up, however, includes closed quarantine, production of pest-free seeds, field inspection and cleaning, laboratory testing and seed treatment and disposal of risky samples. Since the scope of this paper is limited to experimental materials, embargoes and unrestricted shipments are not considered. As a result, importation of maize seed into Ethiopia (Table 1) so far considered the prior approval of import permits by the regulatory body Ministry of Agriculture (MoA) and each imported consignment was accompanied by a world standard phytosanitary certificate from the source country based on inspections of competent experts. All prerequisites to be fulfilled in the certificate are specified in the import permit and hence samples should be treated accordingly during and after shipments. After verification and release of the consignments for post-entry follow-up by EIAR, all samples were subjected to a series of inspection and detection procedures, although still not adequate. Cleaning, sorting and disinfection were carried out to salvage safe germplasm materials whenever possible and unsafe ones were destroyed to avert risk.

Pest Risk Analysis (PRA)

In actual practice, on a worldwide scale, issues of inadvertent importation of potentially hazardous pests into new areas arise in relation to several dimensions that include biological, economic, political and social scopes [7]. These are factors determining the

entry status of an item, in our case maize seed, and subsequent post-entry follow-up. When only one of these factors, especially the biological factor, is in use to determine the entry status of items, the activity ought to be based on PRA. PRA is a thoughtful process whereby the entry status of maize plant, plant products, cargo, baggage, mail, common carriers, etc. is based on calculated risk of inadvertently introducing hazardous pests/pathogens with the maize as transported by man [7,6]. Therefore, PRA is an indispensable process when importing maize seed into this country and hence is carried out for maize seed importation into Ethiopia from eleven major source-countries using information from the CABI Plant Protection Compendium [8,9] and current information on pests and diseases of maize in Ethiopia [10-12].

PRA has three phases including (i) initiation, (ii) risk assessment, and (iii) risk management. The initiation phase starts with the request of a client to import plant materials for planting. At this stage, plant quarantine specialists initiate PRA with some details described in the request form. If no specific pest species were a concern in this import request, a pathway analysis of PRA options was perceptibly followed and the pathway details considered in this paper are (i) country of origin, including Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt, (ii) importing country is Ethiopia, (iii) crop is *Zea mays* L., and (iv) commodity type is seed. Risk assessment considers two areas of information that eventually determine the pest balance of the country. Pest balance, in this case, is the list of pests that are present in the country of origin minus the list of pests widely distributed in the importing country. From this, two pest categories including those potentially requiring phytosanitary measures and those pests excluded from the risk assessment are determined. This information enables us to differentiate pests of quarantine concern to the country (Table 2). Listing pests and determining the mode of transmission and dissemination from source to destination are important and a useful tool to decide import permissions or on the type and level of post-entry follow-ups. New pest records from all directions are essentially important for conducting a sound PRA. Pests recorded on the host plant, liable to be carried on the commodity and absent in the importing country are considered for phytosanitary measures (Table 2) while pests recorded and widely distributed in the importing country were excluded from risk assessment (Table 3). Currently *Spodoptera frugiperda* (fall armyworm), *Papaipema nebris* (stalk borer) and *Puccinia polysora* (American corn rust) has been recorded in Ethiopia on maize as a major disease [13-15] and hence the pest list considered in CABI Plant Protection Compendium was modified for this PRA. Generally, the order of pests in PRA follows as; (i) insect, (ii) fungus, (iii) bacteria, (iv) viruses, (v) nematodes, and (vi) weeds.

A total of 18 pests (including 12 arthropods, 3 fungi, 1 bacterium, 1 virus and 1 *Spiroplasma*) are of quarantine concern when importing maize seed into Ethiopia from the eleven major germplasm source-countries considered in this PRA (Table 2). The number and species of pests of quarantine concern for the country vary depending on country of origin the highest being in Mexico consisting of 11 pests followed by India (7 pests) and then Colombia (6 pests). The rest consists of only 1-5 pests of quarantine importance for Ethiopia. Risk elements considered for this analysis included climate-host interaction, host-range, dispersal potential (population dynamic and epidemiology), and possible economic and environmental impacts. Accordingly, detection protocols and phytosanitary measures are given for each pest or category in the following sections.

Table 1: Maize planting materials imported into Ethiopia in the last 10 years (2011-2020).

Year	Zimbabwe	Kenya	Mexico	India	Nigeria	Norway	Colombia	Zambia	Egypt	Thailand	South Africa	No. of samples
2011	3802	286	1534	5	2515	16	108	0	0	0	0	8,266
2012	1375	1879	0	2384	1054	0	0	0	0	0	0	6,692
2013	3603	0	184	0	791	0	0	0	0	0	0	4,578
2014	420	0	91	0	0	0	0	0	0	0	0	511
2015	11	0	742	3	0	0	0	6	5	0	0	767
2016	4	0	10747	0	3760	0	0	0	0	0	0	14,511
2017	382	0	138	5	378	0	0	0	0	15	384	1,302
2018	1656	25	49	0	112	0	0	0	0	0	62	1,904
2019	872	802	160	0	4	0	0	0	0	0	10	1,848
2020	23	0	614	0	5252	0	0	0	0	0	0	5,889
sum	12148	2992	14259	2397	13866	16	108	6	5	15	456	46,268
Average	1214.8	299.2	1425.9	239.7	1386.6	1.6	10.8	0.6	0.5	1.5	45.6	4,626.80

Source: Holetta Agricultural Research Center [2]

Table 2: Pests of maize potentially requiring phytosanitary measures when imported into Ethiopia.

No.	Pest	Mexico	Colombia	Zambia	India	Norway	Kenya	Egypt	Thailand	Nigeria	Zimbabwe	South Africa
1	<i>Aceria tosichella</i> (wheat curl mite)											
2	<i>Anaphothrips obscurus</i> (grass thrips)	x	x		x			x				
3	<i>Carpophilus hemipterus</i> (dried-fruit beetles)	x			x	x		x		x		x
4	<i>Chaetocnema pulicaria</i> (corn flea beetle)											
5	<i>Glischrochilus quadrisignatus</i> (four-spotted sap beetle)											
6	<i>Helicoverpa zea</i> (American cotton bollworm)	x	x									
7	<i>Lygus lineolaris</i> (tarnished plant bug)	x										
8	<i>Mythimna unipuncta</i> (rice armyworm)	x	x		x							
9	<i>Ostrinia nubilalis</i> (European maize borer)					x		x				
10	<i>Peridroma saucia</i> (pearly underwing both)	x	x			x						
11	<i>Phyllophaga</i> (white grubs)	x			x							x
12	<i>Sesamia nonagrioides</i> (Mediterranean corn stalk borer)									x		
13	<i>Cochliobolus heterostrophus</i> (southern leaf spot)	x	x	x	x		x	x	x	x	x	x
14	<i>Fusarium</i> spp. (seedborne fusaria)	x			x	x	x	x	x	x	x	x
15	<i>Mycosphaerella zeae-maydis</i> (yellow leaf blight)						x					x
16	<i>Pantoea stewartii</i> (bacterial wilt of maize)	x			x			x				
17	<i>Spiroplasma kunkelii</i> (corn stunt spiroplasma)	x	x									
18	Maize chlorotic dwarf virus					x				x		
Total number of pests for each country		11	6	1	7	5	4	5	4	5	2	5

Source: [8-12].

Table 3: Pests excluded from the pest risk analysis conducted for maize seed importation into Ethiopia during 2009 and 2021.

No.	Pest	Mexico	USA	Nigeria	Burkina Faso	Uganda	Kenya	Zimbabwe	South Africa
1	<i>Delia platura</i> (bean seed fly)	X	X			X	X	X	X
2	<i>Rhopalosiphum maidis</i> (green corn aphid)	X	X	X		X	X	X	X
3	<i>Glomerella graminicola</i> (red stalk rot of cereals)	X	X	X	X	X	X	X	X
4	<i>Puccinia sorghi</i> (common rust of maize)	X	X	X		X	X		X
5	<i>Setosphaeria turcica</i> (maize leaf blight)	X	X	X	X	X	X	X	X
6	<i>Sphacelotheca reiliana</i> (head smut of maize)	X	X	X	X	X	X	X	X
7	<i>Stenocarpella maydis</i> (ear rot of maize)	X	X	X		X	X	X	X
8	<i>Ustilago zae</i> (common smut of maize)	X	X	X		X	X	X	X
9	<i>Puccinia polysora</i> (American corn rust)	X	X	X		X	X	X	X
10	Cucumber mosaic virus (cucumber mosaic)	X	X	X		X	X	X	X
11	Maize dwarf mosaic virus (dwarf mosaic)	X	X	X	X	X	X	X	X
12	Sugarcane mosaic virus (mosaic of abaca)	X	X	X		X	X	X	X
13	<i>Spodoptera frugiperda</i> (fall armyworm)	X	X			X			
14	<i>Papaipema nebris</i> (stalk borer)		X			X			
	Total number of pests for each country	13	14	11	4	14	12	11	12

Source: [8-14].

PROTOCOLS FOR INSPECTION AND DETECTION

Maize seed dry inspection

Dry seed inspection is the primary step in seed study for pests and all samples pass through this process. Usually, visual or aided examination of dry seeds using magnifiers or binocular microscope provides adequate information on the presence of pest infestation or symptoms of seed infection from many pathogens. Some pathogens and internal infestation by arthropods, however, need special detection methods to confirm pest infestation. In this case, appropriate detection methods should be specified for each specific pest considering its requirements and conditions.

Detection of Arthropods

Twelve arthropod pests (Table 2, No.1-12) are of quarantine concern for Ethiopia. Inspection, as described above, is just enough to confirm external infestation. Internal infestation, however, needs further detection methods that include dissection, incubation, staining or X-ray. For maize seeds, dissection and/or incubation methods could provide adequate information and are dependable methods, although time consuming. Specific conditions of the pest or group of pests determine the conditions of incubation.

Detection of Fungi

Three fungi (*Cochliobolus*, *Fusarium* and *Mycosphaerella*; Table 2, No. 13-15) are of quarantine concern in imported maize seed to Ethiopia. Blotter and agar plate methods could generate reliable data at a reasonable cost. The blotter method is rather easy and in the agar plate method, general media such as Potato Dextrose Agar, Malt Extract Agar or any seed extract enriched dextrose-agar and incubated at temperature between 20°C and 25°C could serve the purpose.

Detection of the Bacterium

One bacterium (Table 2, No. 16) bacterial wilt of maize (*Pantoea stewartii*) is of quarantine concern in maize seed imported into the country [1,9]. Many workers [15] recommended enzyme linked immune sorbent assay (ELISA) for detection of this bacterium. However, the agar plate method could generate data at a reasonable cost. Specifically, the medium should contain important nutritive substances and eventually be incubated at 25°C.

Inspection and detection of viruses and *Spiroplasma*

One virus (Maize chlorotic dwarf virus) and one *Spiroplasma*

(*Spiroplasma kunkelii*) are of quarantine concern in maize seed imported into Ethiopia (Table 2, No. 17–18). Inspection could not help much for this group of agents. Thus, detection methods including grow-out test in the greenhouse and field inspection with rigorous rouging for cleaning could help in reducing the risk of establishment in the country.

Inspection of weed seeds and identification

Maize seed should be free of any weed seed to avoid their establishment in the country. Visual and/or aided inspection of dry seeds using magnifiers or binocular microscopes provide adequate information on presence of any weed seed, but identification of the weed species requires growing weed seed under well-protected situations in the greenhouse. Weed species of quarantine concern to Ethiopia should be handled with great care and responsibility.

Phytosanitary measures against pests of quarantine concern

Phytosanitary measures include all risk management aspects that individuals and/or groups operate at different levels to safeguard the country from hazardous alien species. Therefore, the following points should be considered during the process of importation:

1. Specify seed treatment with effective insecticides against arthropods and effective fungicides against fungi of quarantine concern for Ethiopia in the import permit form and provide this information to your source before shipment.
2. Inspect consignments (samples and containers) for pest infestation during arrival at entry ports (land, sea or airport) and destroy infested parcels, bags and boxes by appropriate methods. The National Plant and Animal Health and Quality Inspection Service of the MoA, Ethiopia, are responsible for these measures and all must cooperate for the success of this important control measure.
3. After the release of consignments by the MoA, seed samples should be inspected thoroughly and suspected samples subjected to appropriate detection methods described earlier. Consider cleaning, sorting, physical treatment, chemical treatment, etc. of suspected samples to salvage clean and safe maize materials. A list of prohibited weed species and other articles is given in the Plant Quarantine Regulation of the Council of Ministers Article 4/1992, Ethiopia or in the plant Quarantine for NARS [5]. If salvaging through these methods does not seem to be feasible, then the samples should be destroyed together with containers using an incinerator.
4. All samples imported for research should usually pass through post-entry follow-ups depending on the situation [5]. Different measures and handling are specified for quarantine pests of maize in the following post-entry follow-ups section.

Post-entry follow-ups

Receiving maize samples for post-entry follow-ups involves inspection and detection of pests of quarantine concern in maize seed. Samples are subjected to appropriate phytosanitary measures described above and post-entry measures described hereafter,

depending on the specified conditions required. These measures and practices include:

Field inspection and cleaning

Maize imported for research purposes is planted in the first season only at Ambo (for highland types), Bako (mid-altitude sub-humid), or Werer and Melkasa (low moisture stress areas) where field inspection and cleaning is done by crop protection specialists. At this stage, rigorous rouging and destruction of suspected pest, refuses or any strange plant in the nursery are important activities. Both viruses and the *Spiroplasma* show conspicuous symptoms and hence are safely cleared during field inspection and cleaning.

Growing-on test

Some samples might be very small and suspected for bacterial wilt of maize by *Pantoea stewartii*. ELISA was the only recommended detection method for this bacterium, however, some laboratories use the growing-on test. In this case, samples are tested in the greenhouse under controlled conditions where seeds from only healthy plants are released for planting in the next season.

Seedling symptom test

Some maize samples may be very small and suspected for the virus Maize Chlorotic dwarf virus and/or Corn stunt *Spiroplasma* (*Spiroplasma kunkelii*) that are of quarantine concern to the country and hence such samples are tested in the laboratory and seedlings are observed for infection with only clean ones transplanted to give clean seed.

Grow under controlled conditions

Some maize samples might be very small and suspected for any number of serious pests including arthropods, fungi, bacteria, or viruses which are of quarantine concern to the country. These are tested in the laboratory/greenhouse/cold-frame/quarantine fields and then clean seeds are released for planting in the next season.

DISCUSSION AND CONCLUSION

Maize improvement program in Ethiopia utilizes a large amount of germplasm from external sources, seed samples were imported from different countries including Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt from 2011 to 2020. During maize importation, regular inspection was carried out at Holetta Agricultural Research Center (HARC) of the EIAR for freedom from pests that include insects, pathogens and weed seeds. This review provides a concerted approach for effecting pre- and post-entry regulatory measures for maize importation into Ethiopia based on pest risk analysis (PRA). It also provides protocols for inspection and detection, and phytosanitary measures for potentially risky pests to safeguard the country. A total of 18 pests (including 12 arthropods, 3 fungi, 1 bacterium, 1 virus and 1 *Spiroplasma*) are of quarantine concern when importing maize seed into Ethiopia from the eleven major germplasm source-countries considered in this PRA. The number and species of pests of quarantine concern for the country vary depending on country of origin the highest being in Mexico consisting of 11 pests followed by India (7 pests) and then Colombia (6 pests). The rest consists of only 1–5 pests of quarantine importance for Ethiopia. Three fungi (*Cochliobolus*,

Fusarium and *Mycosphaerella*) and one bacterium bacterial wilt of maize (*Pantoea stewartii*) are of quarantine concern in imported maize seed to Ethiopia. Some maize samples may be very small and suspected for the virus Maize Chlorotic dwarf virus and/or Corn stunt *Spiroplasma* (*Spiroplasma kunkelii*) that are of quarantine concern to the country and hence such samples are tested in the laboratory and seedlings are observed for infection with only clean ones transplanted to give clean seed. Accordingly, this review describes quarantine precautions in the import control scenarios and presents PRA based on pathway analysis for importing maize seed into Ethiopia from eleven major source-countries including Mexico, Kenya, Zimbabwe, India, Norway, Colombia, Thailand, Zambia, South Africa, Nigeria and Egypt. Finally, Importers of maize into Ethiopia need to strictly consider the information provided in this paper before, during and after arrival of maize samples for research.

FUTURE CONSIDERATIONS

Importers of maize into Ethiopia need to strictly consider the information provided in this paper before, during and after arrival of maize samples for research. Listing of pests, especially pathogens causing maize diseases should follow scientific methods that eventually confirm their existence and depict their distribution in the country. All aspects of risk management (phytosanitary measures recommended) suggested in this review should be implemented and post-entry quarantine should be considered an essential criterion for advancing imported maize into subsequent nursery stages. Plant protection specialists should be involved in the early growth stages of imported maize samples and subsequent follow-ups for at least two seasons of nursery management to reduce the risk of pest establishment. There should be adequate cooperation with National Quarantine System that provides guidance and authority to this important issue with shared responsibility to safeguard national agriculture and the environment. A network among scientists working in maize improvement and staff in the extension system in maize growing areas should be established to obtain timely pest assessment records and feedback on strange pest occurrences.

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