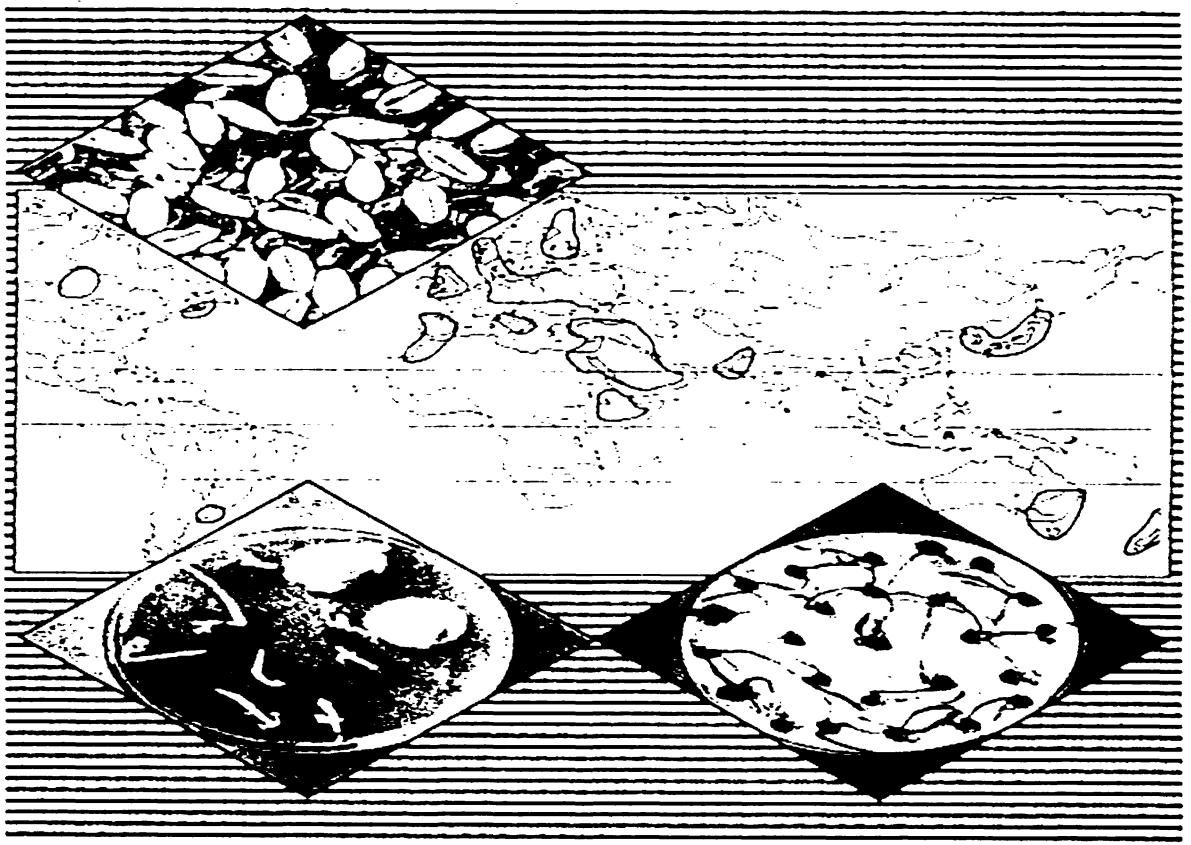
ISSN 0259-2517

Quarantine for seed

FAO
PLANT
PRODUCTION
AND PROTECTION
PAPER

119



Food and Agriculture Organization of the United Nations

Quarantine for seed

Edited by
S.B. Mathur
Danish Government Institute of Seed Pathology for Developing Countries
Hellerup, Copenhagen, Denmark
and
H.K. Manandhar
Central Division of Plant Pathology
Nepal Agricultural Research Council
Khumaltar, Latipur, Nepal

Proceedings of the Workshop on Quarantine for Seed in the Near East, held at the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syrian Arab Republic, 2 to 9 November 1991

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FAO FIAT PANIS

Rome, 1993

Chickpea and Groundnut Seed-borne Diseases of Economic Importance: Transmission, Detection and Control

M.P. HAWARE, D.V.R. REDDY and D.H. SMITH

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India.

Introduction

With the establishment of International Agricultural Research Centers (IARC) the international exchange of seed has greatly increased. For example, during 1989 ICRISAT exported 15164 samples of chickpea seed and 6075 samples of groundnut seed to 41 and 37 countries, respectively. There is always a risk that pathogens can be transmitted by seed.

Seed-borne fungi, bacteria and viruses result in yield losses, reduction in seed germination, increased risk of deterioration in storage, and harmful effects on humans and animals because of toxic metabolites of certain mole fungi.

We need to approach the problem of seed-borne diseases rationally and develop a research programme that is scientifically sound. Not all microorganisms carried in or on the seed are seed-borne pathogens. Before developing a disease management programme it is important to know which of the seed-borne diseases are capable of causing reduction in yield and quality and what is the importance of seed-borne inoculum in spread of the disease.

In this paper we describe the more important seed-borne diseases of chickpea and groundnut, and discuss their transmission, detection, and control.

Seed-borne diseases of chickpea caused by fungi

Chickpea (*Cicer arietinum*) is an important grain legume crop in the Indian subcontinent, Middle East, Northern and Eastern Africa and Central and South America. It is an important source of protein. According to the FAO Production

Submitted as Conference Paper No. 1993 of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324, India

Year Book (1988), chickpea was planted on an area of 10.61 million hectares in 1986 and production was nearly 7 million tonnes. Though the overall yield potential of present day cultivars is over 3000 kg ha^{-1}, the average productivity is only about 700 kg ha^{-1}.

About 45 fungal diseases have been reported on chickpea from different parts of the world (Nene *et al.*, 1989). Of economic importance are Fusarium wilt (*Fusarium oxysporum* f.sp. *ciceri*), dry root rot (*Rhizoctonia bataticola*), Ascochyta blight (*Ascochyta rabiei*) and Botrytis gray mold (*Botrytis cinerea*). Of the numerous diseases recorded, very few are seed-borne. Haware *et al.* (1978) described the seed-borne nature of *F. oxysporum* f.sp. *ciceri*, and seed-borne diseases caused by this species and by *A. rabiei*, *B. cinerea*, *Colletotrichum dematium* and *Alternaria alternata*. Methods for their detection in seed were described in a technical bulletin (Haware *et al.*, 1986). This bulletin is primarily intended for seed production, seed certification, and plant quarantine personnel.

Fusarium wilt

The disease has been reported from several countries. Early infection sometimes kills plants, resulting in total yield loss. In India, it is estimated to cause a 10% annual yield loss. The fungus is a vascular pathogen and is soil- and seed-borne. Seed-borne infection is usually present in seeds harvested from plants which wilt after pod formation. Seeds from wilted plants are generally small, wrinkled, and discoloured. Thus diseased seed can be detected visually, but a seemingly normal seed may also harbour the pathogen. Therefore, it is important to test the seed for the presence of the fungus. Haware *et al.* (1978) showed that the fungus was present in the hilum region of the seed in the form of chlamydospore-like structures. These structures were thick-walled, spherical, closely packed, and connected by hyphal cells. Chickpea cultivars differ in the extent of yield loss and seed infection (Haware and Nene, 1980). The most common methods of pathogen dispersal are apparently by seed and soil.

For detection, 400 seeds are surface-sterilized by immersing them for 2 min in 2.5% aqueous solution of sodium hypochlorite. Seeds are then plated onto modified Czapek-Dox agar (10 per plate) and incubated at 20°C for 8 days in a diurnal cycle of 12 h of near-UV light followed by 12 h darkness. The white cottony mycelium of *F. oxysporum* f.sp. *ciceri* can be observed emerging from the seed (Haware *et al.*, 1986). A seedling symptom test should be employed if an agar medium is not available. Surface-sterilized seeds are sown in soil or fine riverbed sand in pots. These pots are kept in a growth chamber or in a glasshouse at 25°C in a diurnal cycle of 12 h light and 12 h darkness. Seedlings should be monitored for wilt symptoms until at least 40 days after sowing (DAS). The seedlings from infected seeds generally wilt between 15 and 25 DAS. The fungus can be isolated from roots. The wilt count closely agrees with the number of colonies detected on selective medium (Haware *et al.*, 1978).

Ascochyta blight

It is an important disease of chickpea in Pakistan, West Asia, and Northern Africa. In Pakistan, about 70% of the crop was lost to the disease in 1979 and in 1980 (Nene, 1982). It also appeared in epiphytotic form in parts of Punjab and Haryana States of India during 1980 and 1981. For the first time, in 1983, Kaiser and Muehlbauer (1984) reported a trace to a high incidence of blight in germplasm evaluation trials at Pullman, USA. Seventy-seven of 125 accessions tested were affected. Cool, wet weather during June and July favoured infection and spread of the pathogen. According to these authors, the pathogen was introduced into the USA on seed imported from Syria and/or India.

The most common and effective method of dissemination of *A. rabiei* appears to be in seed. Infected seeds are small, wrinkled, and have dark brown lesions of various shapes and sizes on their testae. Pycnidia are found in deep lesions on these seed. If pods are infected at maturity, a seemingly normal seed may show only slight discoloration on the surface but may harbour the pathogen.

For seed health testing, potato dextrose agar containing 1 g Dicrystiem-S per litre of medium is suitable. Seeds must be surface-sterilized by soaking them in a 2.5% aqueous solution of sodium hypochlorite for 2 min. *A. rabiei* is slow-growing and if surface contaminants on the seed are not killed, the pathogen may not be detected. Petri plates, each containing 10 seeds, are incubated in diurnal cycles of 12 h near-UV light and 12 h darkness at 22°C for 10 days. The colonies of the fungus grow slowly on seed and are creamy white with black centres.

In the seedling symptom test, seedling emergence is not necessarily affected by seed infection. Indeed, the test does not give a reliable estimate of seed infection because, in many seedlings, the emerging shoots escape fungal contact and thereby no infection of *A. rabiei* is detected.

Control

Host resistance. Sources of high level resistance to the wilt disease are available. Some of these genotypes have resistance to other diseases including dry root rot, black root rot, Botrytis gray mold, Ascochyta blight or Sclerotinia blight. Cultivars including Avrodhi, JG 315, BG 2368 and ICCC 32, ICCV 2 and ICCV 10 are resistant to wilt disease.

For Ascochyta blight, resistance sources both in kabuli and desi germplasm have been identified. Blight resistant cultivars (ILC 3279 in Syria; C 543 and G 688 in India; CM 72 in Pakistan) have been released in different chickpea growing countries. Because of pathogen variability in *A. rabiei*, resistant cultivars are susceptible to some populations of the pathogen.

Cultural practices. Since the wilt pathogen survives in soil for more than 6 years, crop rotation is not effective for disease control. Intercropping, plant population levels, and fertilizer application had no effect on wilt incidence (Zote *et al.*, 1986).

In seeds from plants which wilt after pod formation, seed-borne infection is commonly observed. Hence roguing of wilted plants (at least for seed purposes) at harvest reduces the number of infected seeds. Use of inoculum-free seed reduces the probability of spreading the pathogen to new areas.

A. rabiei can survive in diseased crop debris on or near the soil surface for two years (Luthra *et al.*, 1935). Crop rotation can eliminate this source of primary inoculum. In order to neutralize this inoculum source the dead plant debris should be removed or ploughed deep. This would probably eliminate the production of the perfect stage of the pathogen in the overwintered crop debris. Viable ascospores can be dispersed for long distances on air currents and thus may introduce the pathogen to new areas (Trapero-casas and Kaiser, 1987). Sexual reproduction could also contribute to increased variability of the pathogen.

Seed treatment. An 0.1% Ceresan^R solution seed treatment followed by a seed treatment with 0.2% thiram or 0.2% PCNB has been reported to suppress wilt development. Bavistin^R seed treatment at 2.5 g kg^{-1} seed rate was also effective in pot experiments (Shukla *et al.* 1981). Seed dressing with Benlate T^R (30% benomyl + 30% thiram) at 1.5 g kg^{-1} of seed eradicated the seed-borne inoculum (Haware *et al.*, 1978) of chickpea fusarium wilt. A Calixin M^R (11% tridemorph + 36% maneb) seed treatment has eradicated the seed-borne inoculum of *A. rabiei* (Reddy *et al.*, 1982). The slurry seed treatment at 2.5 g kg^{-1} with Calixin M^R, Calixin M^R + thiram (1:1), Calixin M^R + Bavistin^R, and Bavistin^R + thiram (1:3) when tested by the blotter-method apparently eliminated the seed-borne inoculum. But when plated on Oat-meal agar, the fungus was isolated from 4-6 percent of treated seed (*A. rabiei*) as compared with 30% recovery from untreated seed. However, Calixin M^R, Calixin M^R + thiram, and Calixin M^R + Bavistin^R treatments significantly reduced seedling vigor. Bavistin^R + thiram seed treatment was best for eradication of seed-borne inoculum, significantly increasing the seed germination and seedling vigor (Tripathi *et al.*, 1986). Thiabendazole seed treatment (3 g ka^{-1} seed) is more effective and safer than using Calixin M^R (Reddy and Kababeh, 1984). Treatment of seed with an effective fungicide would control the disease most economically allowing free international distribution of seed and reducing the danger of introducing the pathogen into new areas.

Seed-borne diseases of groundnut caused by fungi, nematodes, and bacteria

The groundnut or peanut (*Arachis hypogaea*) was originated in South America, but it is now widely cultivated in tropical and subtropical areas of six continents. The groundnut has high oil and protein contents. Haulms are fed to domestic animals in many countries.

A world list of groundnut diseases was recently published by Subrahmanyam *et al.*, (1990). There are over 60 fungal diseases, 17 viral diseases, one bacterial disease,

one disease caused by a Rickettsia-like organism, one disease caused by a mycoplasma-like organism, 10 nematode diseases, and two phanerogamic parasites.

Numerous fungi have been isolated from groundnut seed. The fungi frequently associated with seed rotting and seedling diseases are, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* spp., *Lasiodiplodia theobromae*, *Macrophomina phaseolina*, *Penicillium* spp., *Pythium* spp., *Rhizopus* spp., *Sclerotinia minor* and *Sclerotium rolfsii*. Most of these pathogens occur widely in many groundnut-growing areas of the world.

Several seed protectant fungicides are used either as a single fungicide or as mixtures of two or more fungicides, depending on the spectrum of fungal pathogens in different groundnut production areas of the world. Formulations of captan, carboxin, DCNA, ethazol, maneb, Metalaxyl, PCNB and thiram are recommended for application to groundnut seed.

Most groundnut seed-rotting pathogens can be detected by plating surface sterilized seeds on potato dextrose agar, but selective methods are available for isolation of species of *Aspergillus*, *Fusarium*, *Macrophomina*, and *Fusarium*.

Sclerotinia minor, the causal agent of Sclerotinia blight of groundnuts, has a wide host range (Akem and Melouk, 1990; Porter *et al.*, 1989). However, since the current distribution of Sclerotinia blight of groundnut is limited, the distribution of seed produced in fields infected with *S. minor* should be avoided. Sclerotia of *S. minor* commonly form within pods and occasionally within seeds. Therefore, seed protectant fungicides may not be effective. Sclerotia of *S. minor* can survive for several years in groundnut fields. This is another reason for avoiding distribution of seed produced in infested fields.

Ditylenchus destructor is an important nematode of groundnut in South Africa (De Waele *et al.*, 1989). The nematode attacks pegs, pods, and seeds. Infested seeds are shrunken. Micropyles are dark brown to black. Testae are flaccid and easily removed, and vascular strands in the testa are dark coloured. The inner layer of the testa has a yellow discoloration. Infected embryos are usually olive green to brown. Detection of this nematode can be done by placing groundnut seed in water for 24 hours. The nematodes can then be observed with a stereoscopic microscope.

We are not aware of measures that can be used to control this nematode either within or on groundnut seeds

Mahmud and Middleton (1990) reported seed transmission of *Pseudomonas solanacearum* the causal agent of bacterial wilt. Some harvested groundnut pods from infected plants were discoloured and some pod rot was observed. No symptoms were observed on pods collected from healthy plants. Discoloration of the seed coat and cotyledon was observed on some seed obtained from infected plants. Discoloration of the embryo was rarely observed. When infected seeds were planted, the incidence of wilting was 5 to 8% at 2 to 4 weeks after planting.

P. solanacearum can be detected using the SPA medium (Hayward, 1964) or the TZC medium (Kelman, 1954). Colonies of bacterium are fluidal and irregular in shape and white or pink centres that darken with age. Avirulent colonies are round butyrous and uniformly red even at the early stage of growth.

Seed-borne virus diseases of groundnut and chickpea

Several economically important groundnut diseases are caused by seed-borne viruses. However, only one seed-borne virus of minor importance has been reported on chickpea (Kaiser *et al.*, 1990). Of the plant viruses which infect groundnut under natural conditions (Reddy, 1991) at least five are seed-transmitted (Tables 1 and 2). Infection of gametes is necessary to facilitate seed transmission of viruses. In the case of peanut mottle (PMV) and peanut stripe (PStV) viruses, infected seed serve as the primary source of inoculum.

Despite availability of highly sensitive and reliable methods for detection of seed-borne viruses, these techniques are not widely used in developing countries. Thus in many countries these viruses may not be detected with current inspection methods. Seed-borne viruses can be detected either by a direct method (growing-on tests) or by indirect methods (infectivity assays, serological tests, and complementary DNA probes). For growing-on tests seedlings are raised in sterilized soil in a greenhouse. Viruses which produce macroscopic symptoms can be easily recognized, but those that produce no overt symptoms escape in this test. Since diagnostic hosts are currently available for all seed-transmitted viruses in groundnut (Table 2), infectivity assays can be used for virus detection. They are recommended for use in countries where facilities for performing serological tests and nucleic acid hybridization tests are not available. Infectivity assays are especially valuable for virus detection in plants exhibiting no symptoms in growing-on tests.

Of all serological tests currently available for the detection of groundnut viruses, enzyme-linked immunosorbent assay (ELISA) is the most preferred assay method. ELISA methods for detection of viruses in groundnut seed have been described (Sudarshana *et al.*, 1990).

All currently known seed-transmitted groundnut viruses contain single stranded RNA. It is possible to produce complementary DNA (cDNA) probes which can be used in a variety of nucleic acid hybridization tests. cDNA probes have been used for detecting PMV and PStV in groundnut seed (Bijaisoradat and Kuhn, 1988). Nucleic acid hybridization tests need highly perishable enzymes and elaborate laboratory facilities. Until non-radioactive cDNA probes (Roy *et al.*, 1988) are made available at affordable costs to research workers in developing countries cDNA probes will probably not be utilized for routine virus detection in seed.

Table 1. Features for identification of seed-transmitted viruses of groundnut.

Virus and virus group	Particle morphology	Serological reactions positive	Serological reactions negative
Peanut mottle potyvirus	Flexuous rods 740-750 nm length 13 nm width	Adzuki bean mosaic	Bean yellow mosaic, Groundnut eyespot, Peanut green mosaic, Peanut stripe
Peanut stripe potyvirus	Flexuous rods 752 nm length 13 nm width	Black eye cowpea mosaic Clover yellow vein Soybean mosaic	Bean yellow mosaic, peanut mottle
Peanut clump furovirus	Two rod shaped particles 245 nm length 160 nm length 24 nm width	Exist as several serologically distinct isolates. Cross reaction occurs among some isolates	Beet necrotic yellow vein, Potato mop top, Soil-borne wheat mosaic
Peanut stunt cucumovirus	Spherical 25-30 nm diameter	With several peanut stunt virus isolates from USA	-*
Cucumber mosaic cucumovirus	Spherical 28 nm diameter	Several isolates of cucumber mosaic and peanut stunt viruses	-*

* Data not given because of their limited value for identification.

The presence of serologically different isolates for viruses such as peanut clump (PCV) may pose problem for using serological techniques for virus detection in quarantine. Thus broad-spectrum monoclonal antibodies and cDNA probes have immense potential for virus detection in seed. Research on production of non-radioactive cDNA probes for PCV is in progress at ICRISAT. The two widely distributed seed-borne viruses, PMV and PStV, are potyviruses and it is possible that other potyviruses may transmit through groundnut seed. Thus it is also essential to produce antibodies which can detect a large number of potyviruses. Recently Shukla and Ward (1989) reported that polyclonal antibodies can be produced for the core region of viral polypeptide, which is highly conserved in different potyviruses. Research on production of polyclonal antisera, for the core region of potyvirus polypeptides, for detection of several potyviruses is in progress at ICRISAT.

Since control measures to eradicate the seed-borne virus inoculum are not available, it should be ensured that only virus-free seeds are used in germplasm exchange. Genotypes in which the virus is not seed-transmitted have been identified

in case of PMV. Efforts are being made to develop agronomically acceptable, non-seed transmitting, high yielding breeding lines.

Table 2. Transmission frequency and host range of seed-transmitted viruses of groundnut.

Viruses	Percent seed Transmission	Diagnostic hosts Local lesions	Systemic
Peanut mottle virus	0.1 to 8.5	*Phaseolus vulgaris* (Topcrop)	*Glycine max* *Pisum sativum*
Peanut stripe virus	0.1 to 30	*Chenopodium amaranticolor* *C. quinoa*	*Glycine max* *Lupinus albus* *Vigna unguiculata*
Peanut clump virus	6.0 to 25	*Chenopodium quinoa* *Phaseolus vulgaris* *Vigna unguiculata* *N. glutinosa*	*Canavalia ensiformis* *Nicotiana clevelandii* *N. edwardsonii*
Peanut stunt virus	0.01 to 0.2	*Chenopodium amaranticolor* *Vigna unguiculata*	*Datura stramonium* *Glycine max* *Nicotiana tabacum*
Cucumber mosaic virus	1.0 to 2.0	*Chenopodium amaranticolor* *Datura stramonium* *Phaseolus mungo*	*Nicotiana tabacum* *Vigna unguiculata*

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