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FIELD INOCULATION TECHNIQUES AND DISEASE RATING SCALE IN MAIZE

BY

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Field inoculation techniques of some diseases of maize (Leaf blights, common rust, downy mildews, *Physoderma* leaf spot, stalk rots banded leaf and sheath blight and ear rots) of major importance in India are described and discussed here. The different methods of rating these diseases under field condition are also included.

INTRODUCTION

Maize crop has been found to be susceptible to as many as 61 diseases under Indian conditions (Payak and Sharma, 1980). Among them, 15 are considered to be of major importance as they are widely spread throughout the country and may cause considerable damage to this crop resulting significant loss in yield. These includes : (a) three foliar diseases, namely (i) *Turcicum* leaf blight *(Exerohilum turcicum)*, (ii) Maydis leaf blight *(Drechslera* state of *Cochliobolus heterostrophus)* (iii) Common rust *(Puccinia sorghi)* ; (b) five stalk rots, namely (i) Bacterial stalk rot *(Erwinia chrysanthemi)*, (ii) *Pythium* stalk rots *(Pythium aphanidermatum)*, (iii) *Fusarium* wilt and/or stalk rot *(Fusarium moniliforme)*, (iv) Late wilt *(Cephalosporium maydis)*, (v) Charcoal rot *(Macrophomina phaseolina)* ; (c) four downy mildews, namely (i) Brown stripe downy mildew *(Sclerophthora rayssiae* var. *zeae)*, (ii) Philippine downy mildew *(Perenosclerospora philippinensis)*, (iii) Sorghum downy mildew *(Perenosclerospora sorghi)*, (iv) Sugarcane downy mildew *(Perenosclerospora sacchari)*, (d) two that affect leaf, sheath/ear, namely (i) Brown spot *(Physoderma maydis)*, (ii) Banded leaf and sheeth blight *(Rhizoctonia solani* f. sp. *saskii)* and (e) one nematode disease (Cyst nematode, *Heterodera zeae*).

Although the incidence of these diseases may be appreciably high in some year under natural field condition their incidence may be sufficiently low, or even they may be absent in the following years due to absence of sufficient inoculum or sources of inoculum to infect maize in the field ultimately leading to the disturbance or failure of the screening programme. In order to avoide and overcome this uncertainity the disease may be induced to this crop in the field by artificial inoculation. There are variaous methods of field inoculation techni-

ques which may be common to one or more than one diseases under a particular set of environment. A detail account of which have been presented and discussed in this paper. The rating scales of these diseases have been also included here. It is hoped that the informations provided here may be useful particularly to the students of plant pathology who are working with maize or some other related crops. Nevertheless, it will also draw considerable interests to others.

Field inoculation techniques :

The various methods of field inoculation for different maize diseases developed by different workes and practiced in the All India Coordinated Maize Improvement Project are described here with or without modifications.

Leaf blights

1. Turcium leaf blight *(Exerohilum turcicum)* and
2. Maydis leaf blight *(Drechslera* state of *Cochliobolus heterostrophus)*. There are three methods of field inoculation procedurse for these two leaf blight pathogens.

(i) *Using suspension of the pathogenic cultures*

The original cultures of the above two pathogens are to be isolated from the leaf lesions after incubating them in moist chamber for 3 to 4 days when new spores would have formed on the surface of the lesions. These newly formed spores are picked up by means of sterile flattened needle under a dissecting microscope and then placed in a droplet of sterile distilled water which is streaked accross the surface of sterile acidified water agar in petriplates. After allowing a few hours for the spores to germinate, they are cut out of the agar and transferred to acidified PDA medium and incubated at 25° ± 1°C for 2 weeks inorder to get the stalk cultures for mass increase of inoculum to be used for field inoculation. When the fungus growth has covered the surface of the agar the cultures are ready for use. About 20 petriplates of cultures are macerated in a Waring blender for 20 to 30 seconds after which it is strained through a layer of muslin cloth to which is added about 5 litres of water to get the stock suspension. This stock suspension is then taken to the field and diluted in a compressed air sprayer (which has not been used for spraying any chemical before) @ 1 litre in about 12 litres of water. 4 litres of stock suspension diluted as indicated above is enough to inoculate about 1,000 plants.

Inoculum should be directed into the whorls of the plants, where it will be retained long enough to permit spore germination. Inoculations should be made at weekly interval for 3 weeks begining when plants are 30 to 45 cms

high. This method is highly useful in establishing heavy infection. Mixed inoculation with these two blight pathogens may be practiced to permit selection against them at a time.

(ii) *Using infected leaf powder*

Inoculum is most easily prepared by using heavily infected leaves collected in the previous season. Only mature leaves should be collected by avoiding the green leaves. These leaves are to be stored in loosely packed gunny bags in a cool and dry place. Prior to inoculation the leaves are ground by milling, or by putting them in tightly woven sacks and beating them into coarse powder of about the coarseness of wheat bran. About 30 bags of blighted leaves will yield about 50 kg of powder enough to inoculate approximately 10,000 plants 4 times.

Inoculation is made by placing a pinch of leaf powder into the whorl of each plant of about 30 to 45 cms high. This is followed by spraying with water using a knapsack sprayer directing the spray into the whorl. The inoculation is repeated 4 times at weekly interval. Leaf powder of both the pathogens may be simultaneously used together for inoculation to permit infection.

(iii) *Using grain culture of the blighted pathogen*

Inoculum is increased on whole sorghum grains of about 1 inch layer in conical flasks. The grains are soaked in water for about 4 to 6 hours after which the excess water is drained off. To this is added potato dextrose just enough to provide a thin coating on the grains. The flasks containing the sorghum grains are then autoclaved at 15 lbs pressure for 20 minutes and seeded with the pathogen. The flasks are then inoculated at 25° $\pm$ 1°C for about 2 weeks when the material becomes ready for inoculation. One teasponful of the grain is mixed with 1/2 litre of water and blended for 120 seconds in a Waring blender. The suspension is collected and diluted by adding 4 parts of water. The diluted suspension is used for inoculation as described before.

Common rust (Puccinia sorghi)

The following two methods may be employed for inoculation of common rust in maize.

(i) *Direct inoculation with uredospores*

As rust is an obligate parasite it is very difficult to grow it on artificial media under laboratory condition for mass increase of inocuium. Although for some specific purpose small amount of inoculum can be grown under laboratory conditions on detached leaf culture, the amount of uredospores obtained by such method is not sufficient enough to be used in large scale for field ino-

culation. Therefore, naturally infected leaves showing large number of uredopustules should be collected for the preparation of inoculum to be used for field inoculation.

The infected leaves thus collected are then macerated in between the two palms of hands dipped under a bucket of water until the water gets sufficiently coloured. The uredospores can also be collected in a butter paper by tapping the severely infected leaves with fingers and then stored in a glass vial or glass tube which can be sealed easily under a flame. The uredospores, thus obtained may stored for a longer period at a lower temperature between 5° to 7°C and can be used for inoculation when required.

Field inoculation should be made with 35-45 cms hight plants by using a knap sprayer. The spore suspension should be sprayed over the plants preferably in the afternoon when the sun becomes mild. The spore suspension must be stirred continuously during spraying as light spores may aggregate together on the upper surface of the suspension. The inoculation may be repeated twice at a weekly interval for getting good result. This method of inoculation is practiced at Dholi. Bihar (Gupta. 1982) may be accepted for use with or without modifications.

(ii) *Use of infector (donor) plants*

Infector plants are to be raised before planting of the test plants. The infector plants should be inoculated with the inoculum as in the previous case for providing the inoculum source. Testing plants are to be planted when inoculation of the infectors is complete. One row of infector plants should be planted first and the next two rows should be covered by the testing plants followed by another row of infector plants. This process may be repeated to cover a desirable area of land in a large testing programme and for large number of testing germplasm. The testing plants may readily get uredospores from the infector plants thus providing for the suitable source of inoculum for rapid infection and spread of the disease.

Downy mildews

1. *Perenosclerospora* downy mildews *(P. philippinensis, P. sorghi* and *P. sacchari* etc.*)*

The different inoculation procedures for *Perenosclerospora* DMS in maize are : (i) Use of soil-borne oospores, usally in a sick piot, (ii) Direct inoculation with conidia and (iii) Conidia supplied from the infector (donor) plants within the field.

Oospore 'Sick plot' are used effectively at the University of Mysore (Safeeulla, 1976) where testing plants are readily infected with the soil-borne oospores.

The limitations of their use at most other places are that (a) oospores are not formed, (b) when formed germination and infection are erratic, (c) infection in a field are irregular and patchy and (d) the result is that oospores are generally unavailable as a source of inoculum.

Most laboratories use conidia for direct inoculation or use donor plants, or a combination of the two methods. The method employed in Thailand as described by Renfro (1982) is practiced at many locations in India with some modifications as detailed below :

The DMS donor plants are established in the field about 2 weeks before planting the test materials, and they are inoculated with conidia 2 to 3 times at weekly interval, the first inoculation being at first whorl stage. The source of inoculum for such inoculation may be from a few to several locations. The donor plants thus inoculated provide the inoculum source for the test plants which may be usually inoculated twice. The methods for collection of conidia and preparation for conidial suspension are as follows :

Sporulating leaves from the fields should be collected at 0200 to 0300 hrs. or harvested diseased leaves in the evening which may be incubated at dark, moist conditions at 20° to 24°C for about 8 to 10 hrs for spores to form. From these leaves the conidia are harvested and the aqueous based conidial suspension with requisite inoculum density (at least 50,000 conidia/ml) is made for field inoculation that may be operated by means of a knap-sack sprayer.

Diseased leaves may also be collected at clear bright days at 1100 hrs or on overcast days at 1300 hrs. The old conidiophores should be washed off and these leaves may be placed in plastic pails containing 4 to 5 cm water in the bottom and to be incubated at 20° to 24°C for 8 hrs. The spores are harvested by washing in water.

2. *Brown stripe downy mildew (Sclerophthora ravssiae* var *zeae)*

Two methods of field inoculation in case of BSDM are widely practiced under Indian conditions. These are :

(i) *Using inoculum powder out of infected leaves*

This method was developed by Singh *et. al.* (1970). The inoculum may be prepared from infected maize leaves, collected during the last season, containing oospores or from the infected leaves supposed to be full of oospores collected from early planting of maize of the season. Mass of inoculum powder from these infected leaves may be prepared in the same way as described earlier. Inoculum should be placed in furrows just before planting in such a manner that seed when planted come to close proximity of the inoculum.

(ii) *Using freshly infected detatched leaves*

Pieces of freshly infected leaves (2 to 3 cm) containing sporangia of the fungus should be placed in the whorls of the seedlings during cloudy weather between 1700 and 1900 hrs on 17, 24 and 30 days after planting (Singh and Renfro, 1971). This method is particularly useful in areas where the disease appears year after year. In areas of low disease incidence, both the methods can be combined to obtain better results.

Stalk rots

1. *Bacterial stalk rot (Erwinia chrysanthemi* corn pathotype)

The syringe inoculation technique as developed by Thind and Payak (1978) may be practiced in case of *E. chrysanthemi* for field inoculation as described here.

Preparation of inoculum

A virulent isolate of the pathogen should be isolated from the small piece of rotten infernode removed aseptically from the infected stalk of the host plant. After rinsing the piece in spirit, it is immediately dipped into $HgCl_2$ solulion (1 : 1000) for 30 seconds and passed through three changes of sterile water. The piece is then cut into two halves with sterilized blade, put into little sterile water and then teased apart with sterile needle. A small quantity of the resulting suspension is then aseptically transferred by streaking out on well dried nutrient agar plates to get individual colories of the bacterial cells. The characteristic colonies are identified after 48 hrs of incubation at 28° ± 1°C and used for subculturing. The inoculum is then increased on nutrient broth for 48 hrs at 28° ± 1°C. The inoculum is diluted 10 times to get desired concentration of the bacterium (approximately 1 x 10^{8-9}/ml.

Inoculation procedure

Inoculation is made at the presilking stage, At first a diagonal hole deep upto the pith, is made in the middle of the 2nd internode from the soil level with the help of a jabber. 1 ml of the bacterial suspension is then injected through that hole by a hypodermic syringe. If necessary, a second inoculation may be made in the 3rd internode. The puncture points are to sealed with lubricating grease to avoid dessication.

2. *Pythium* stalk rot *(Pythium aphanidermatum)*

The tooth pick inoculation technique as described by Diwakar and Payak (1974) is widely practiced for field inoculation of this stalk rot.

The pathogen associated with the disease should be isolated from the infected

stock in PDA medium for getting the stock culture of the pathogen. The inoculum for field inoculation is increased on round sterilised bamboo toothpicks, partially submerged in potato dextrose broth supplemented with 0.1% yeast extract as follows. Round hard bamboo toothpicks of 5.5 cm on length with tapering ends are boiled several times thoroughly in water to remove resin, gum or any other toxic substance that might inhibit the growth of the fungus. After washing they are dried in sun. Keeping the tapering ends upward the dried toothpicks are stacked loosely and submerged in potato dextrose broth in screw-capped or cotton plugged jars and subjected to autoclaving at 15 lbs pressure for 20 minutes. The level of the broth so adjusted that it covers one-third length of toothpicks after autoclaving. The sterilized jars are then seeded with the pathogen and incubated at 28° ± 1°C for 7-10 days. The cottony white mycelial growth will cover the toothpicks and these are used for field inoculation.

The plants are inoculated within 5 to 7 weeks after planting (prior to flowering). About 2 cm deep whole is made in an oblique manner in the centre of a basal internode by means of a jabber. The toothpicks are then inserted into the holes. The round toothpicks thus effictively seal the orifice in the stalk and prevent drying of the stalk tissue.

3. *Charcoal rot (Macrophomina phaseolina)* and

4. *Late wilt (Cephalosporium maydis)*

The methods for preparation of inoculum and field inoculation procedures of charcoal rot and late wilt diseases in maize are similar as in case of *Pythium* rot.

Diseases affecting leaf, sheath/ear

1. *Brown spot (Physoderma maydis)*

Whorl inoculation technique has been found to be most suitable for this disease. This method is practlced at Udaipur, Rajasthan including other locations.

Inoculum is prepared from the infected leaves (fresh or stored for 1 to 2 years) which are cut in small pieces and put in water for thorough moistening and then blended in a Waring blander in tap water for a desirable period and finally passed through muslin cloth to get the sporangial suspension which is then diluted by pouring water to bring the concentration of sporangia upto 5000/ml. This inoculum suspension is filled in small dropping bottles and the plants are inoculated at the susceptible stage of growth (30 ± 10 days) by putting few drops of inoculum in the whorl.

2. *Banded leaf and sheath blight (Rhizoctonia solani* f. sp. *saskii)*

Ahuja and Payak (1978) have described the field inoculation technique for this disease.

The fungus after isolating from the infected plant parts should be grown on soaked barley grains for mass multiplication of the inoculum for filed inoculation. 40 g of barley grains are soaked in water for 24 hrs and then dispensed in 250 ml Erlenmayer flasks after removing excess water and autoclaved at 15 lbs pressure for 20 minutes. 2 to 3 days old growth of the pathonen taken from PDA is homogenised in sterile water and 5 ml of this is added to each flask containing soaked barley grains and incubated at 26° ± 1°C for about 10 days which can be immediately used for inoculation, or can be air dried at room temperature and stored in paper bags or in the flasks themselves at 15°C for subsequent use.

Inoculation of plant is done during the rainy season (July and August) on 30 to 45 days old plants with grain culture (using 4 grains) inserted between ear and sheath at 2nd or 3rd internodal level from soil. Grains placed at junction of sheath and leaf can also create optimum disease level and do not fall away with strong wind or heavy rain.

Ear rots (Diplodia maydis, Rhizoctonia zeae, Aspergillus niger)

Ear base inoculation technique (Das and Chattopadhyay 1979) may be practiced for ear rots caused by the above fungi. The culture is multiplied by growing the fungus on sterile cooked whole oat medium for 4 weeks in the laboratory at 25° ± 1°C in diffused light to get good sporulation in the culture. It is then thoroughly macerated in sterile distilled water and filtered through sterile muslin cloth followed by centrifugation by a low speed hand centrifuge to get the spore suspension after discarding the supernatant liquid. The spore suspension is then diluted with distilled water to a desirable concentration of about 100 spores per low field of microscope to be used for field inoculation.

Inoculation is made by injecting spore suspension through a hole made in the ear beneath the maximum girth. The hole is made by a sterile steel needle at acute angle through the ear length just to pierce through 2 to 3 layers of husk cover. After inoculation the pores are sealed with cellophane tape to avoid dessication, contamination and washing by rain.

Inoculation should be made at the most susceptible stage. It has been observed that the ears become most susceptible during the first 15 to 20 days after full silk (Kaiser and Sengupta, 1979) and therefore, this stage should be considered for field inoculation inorder to get good result.

Disease rating scale in maize

Once the disease has been established after succeseful inoculation it may produce characteristic sysptoms. However, the intensity of infection may vary depending upon the susceptibility or resistance of the inoculated plant. And therefore, the symptoms of a disease may be asessed into different categories mainly

by visual observations depending upon the intensity of infection. This requires a suitable scale for measurement or rating of the disease. The rating scale for different diseases of maize followed in the Indian Maize Improvement Project (Payak, 1975) have been described here for acceptance with or without modifications.

Foliar diseases

The diseases such as (1) *Maydes* leaf blight, (2) *Turcicum* leaf blight, (3) Brown stripe downy mildew *(Sclerophthora rayssiae* var. *zeae)* and (4) Common rust *(Puccinia sorghi)* included here are rated on the basis of 1 to 5 scale consisting of five broad categories designated by numerals/index from 1 to 5. Intermediate ratings between rwo numerals can also be done e. g. 2.5, 3.5 etc. in which total number of classes or categories come to nine. The measurement is based on number of lesions present on a leaf and their subsequent spread. Wherever possible observations on lession types can also be made such as large sporulating wilt type or small chloratic, nonsporulating type. The scale is described below :

Numeral/Disease index	Intensity of infection	Category
1.0	*Very slight to slight infection,* one or two to few scattered lesions on lower leaves.	Resistant
2.0	*Light infection,* moderate number of lesions on lower leaves only.	Moderately, resistant
3.0	*Moderate infection,* abundant lesions on lower leaves, few on upper leaves.	Moderately, susceptible
4.0	*Heavy infection,* lesions abundant on lower and middle leaves, extending to upper leaves.	Susceptiple
5.0	*Very heavy infection,* lesions abundant on almost all leaves, plants prematurely dry or killed.	Highly Susceptible

Downy mildews

The diseases included are (1) Sorghum downy mildew *(Perenosclerospora sorghi),* (2) Philippine downy mildew *(P. philippinensis* and (3) Sugarcane downy mildew *(P. sacchari).* The observations are made from seedling stage to flowering on the percentage basis, that is the proportion of plants showing disease over healthy or those showing no infection. On this basis plants showing different percentage of infection may be classified into following categories.

Plants infected (%)	Category
0 to 20	Resistant
21 to 40	Moderately resistant
41 to 60	Moderately susceptible
61 to 80	Susceptible
81 to 100	Highly susceptible

Brown spot Physoderma maydis)

Scoring for this disease is recommended on percentage basis, or on 1 to 5 scale as described above in case of foliar diseases.

Stalk rots

Incidence of *Pythium* and Bacterial stalk rots are recorded on percentage basis. For Late wilt *(Cephalosporium maydis)*, Black bundle *(Cephalosporium acremonium)*, Charcoal rot *(Macrophomina phaseolina)* and Botrydiplodia stalk rot *(Botrydiplodia theobromae = Lasiadiplodia theobromae)* the scale had ten classes as follows. The measurement is based on the proportion of disease present in the inoculated internode and its subsequent spread.

Numerals/Disease index	Intensity of Infection	Category
1.0	25% of the inoculated internode discoloured.	Resistant
2.0	26 to 50% of the inoculated internode discoloured.	
3.0	51 to 75% of the inoculated internode discoloured.	
4.0	75 to 100% of the inoculated internode discoloured.	
5.0	Discolouration of less than 50% of the adjascent internode.	Intermediate
6.0	Discolouration of more than 50% of the adjascent internode.	
7.0	Discolouration of three internodes.	Susceptihle
8.0	Discolouration of four internodes.	
9.0	Discolouration of five internodes.	
10.0	Plants prematurely killed.	

The word discolouration has been used in a broad sense to include browning, purpling, blackening, hollow pith and weakening of internodes or stalks.

Banded leaf and Sheath blight (Rhizoctonia solani f. sy. *saskii)*

The disease intensity is recorded on the basis of the following rating scale :

Numeral/Disease index	Disease intensity	Category
1.0	Infection on one leaf sheath, lesions one to few, non-coalescent.	Resistant
2.0	Infection on two to three leaf sheaths, lesions few and non-coalescent on 3rd leaf sheath from ground level.	Moderately resistant
3.0	Infection not upto the ear shoot but on more than two leaf sheaths.	Moderately susceptible
4.0	Infection on all leaf sheaths upto the ear shoot but shank not infected.	Susceptible
5.0	Infection present beyond the ear shoot ; reduced ear size, hust leaves bleached and cacked with or without sclerotial development ; kernel formation absent or rudimentary.	Highly susceptible

Ear rots

The ear and cob rots caused by species of *Fusarium, Aspergillus, Diplodia, Botrydiplodia* and *Rhizoctonia* may be rated according to the following scale.

Numeral/Disease index	Severity of infection	Category
1.0	No infection on kernels or tip of the cobs.	Resistant
2.0	Infection on from one to few kernels to $\frac{1}{4}$th portion of the ear.	Moderately resistant
3.0	Infection on kernels on $\frac{1}{2}$ portion of the ear.	Moderately susceptible
4.0	Infection on kernels on $\frac{3}{4}$th portion of the ear.	susceptible
5.0	Infection on kernels on the entire ear with or without infection of the shank.	Highly susceptible

Das and Chattopadhyay (1979) described the amount of ear rot in a variety on the basis of percentage of infected ear as well as by a disease index formulated by Ullstrup (1949) with some modifications as follows :

$$\frac{n_{C0} + n_{C1} + n_{C2} + n_{C3} + n_{C4} + n_{C5}}{N}$$

where n=number of ears in each class ; CO, C1, C2, C3, C4, C5=the class value (0 to 5) for healthy, $\frac{1}{5}$rh rotted, $\frac{2}{5}$th rotted, $\frac{3}{5}$th rotted, $\frac{4}{5}$th rotted and completely rotted ears.

N=Total number of ears in each variety.

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