

COMPARITIVE EFFICIENCY OF DIFFERENT TYPES OF ARTIFICIAL INOCULATION TECHNIQUES FOR PRODUCING EPIDEMICS TO GIBBERELLA EAR ROT IN MAIZE

BY

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Three different methods of artificial ear inoculation in the field namely silk, silk plus husk and oat kernel were studied on different varieties of maize (*Zea mays* L.) with *Gibberella zeae* (Schw.) Petch at Kalimpong Research Station in the Himalayan hills of West Bengal. Silk plus husk inoculation showed better results than two others and a highly positive co-efficient among three different methods were noted.

INTRODUCTION

Gibberella ear rot caused by *Gibberella zeae* (Schw.) Petch is more prevalent in the North eastern parts of India. In India this disease was first reported on stored cobs in Sikkim by Kapoor in 1963 (Kapoor, 1963). The importance and severity of this disease were not fully understood until Das and Chattopadhyay (1984) studied this disease situation on 178 different germplasm for a period of four years at Kalimpong hills of West Bengal which represents an average agro-climatic conditions of North Eastern hill region of India. From the report it was noted that a large number of maize germplasm were affected, infection ranging from 17.34 to 30.90 per cent with an average disease incidence of 22.99 per cent during the four years of study. The fungus appeared to be more prevalent in cooler and more humid region (Ullstrup, 1960). The climatic conditions of Kalimpong hills are congenial for the natural spread and development of this disease incited by *Gibberella zeae*. Even some of released variety like Diara, Amarillo-de-Cuba, Vijay showed high degree of susceptibility (Das and Chattopadhyay, 1984). Hence it was necessary to test the different maize germplasm against this disease under artificial epidemic condition to locate the resistant one. Different techniques were adopted in the past for the purpose (Koehler, 1950, Ullstrup, 1970 Enerson *et al.* 1980a, 1980b). The present investigation was under taken to find out the comparative efficiency of different artificial inoculation techniques used by various workers.

MATERIALS AND METHODS

The following methods of ear inoculation were reported by Koehler (1950), Ullstrup (1970) and Enerson *et al.* (1980a, 1980b).

1. Spraying the spore suspension over the ears with the help of compressed air sprayer.
2. Inoculating the ears with a pressure sprayer or a medicine dropper.
3. By inserting tooth picks over grown with mycelium into the ear shoot before silking.
4. Spray inoculation with spore suspension over the ears and (subsequently covered with wet paper towelling) and water-repellent cap to prevent drying.
5. With infected barley kernel under husk.

The present paper deals with the comparative efficiency of three different artificial inoculation techniques namely

- i) Silk inoculation
- ii) Silk plus husk inoculation
- iii) Oat kernel method

a) Silk inoculation : By pouring a few drops of spore suspension at the cob-tip on silk after inserting the needle of a plastic syringe, 2-3 mm deep on cob-tip. The whole cob was then covered with a plastic bag and tied at the base of the ear with a rubber band for a period of one week to avoid chances of dessication of spores, washing by rain and contamination.

b) Silk plus husk inoculation : By pouring a few drops of spore suspension at the cob-top on silk after inserting by the help of a plastic syringe and also one drop of spore suspension beneath the husk near the cob-tip and subsequently covering with a plastic bag as in silk inoculation.

c) Oat kernel method : By placing an infected oat kernel seeded with mycelium and spores of the fungus with the help of a sterile forcep near the cob-tip under husk.

All types of inoculation were made 5-10 days after 50 per cent of full-silk. One healthy cob per plant was chosen for inoculation.

Seeds of nine different maize germplasms were sown in a randomized block design with two replication having 15-20 plants in each replication at the University Experimental Station, Kalimpong, situated at an altitude of 1070 metre in the District of Darjeeling. Usual cultural practices were followed. No fungicidal or insecticidal applications were made at any phase of plant growth. For artificial inoculation, the fungus was grown in the following manner.

Preparation of inoculum :

For artificial inoculation with oat kernels, the fungus was grown on sterile cooked whole oat in a 250 ml. fruit jar for a period of two weeks in the laboratory at a room temperature (20-24°C). Infected oat kernels were used for inoculation. For inoculation with spore suspension the fungus was grown on acidified potato dextrose agar on petriplate for a period of two weeks at room temperature (20-24°C). The over-grown fungal spores were collected by scraping with the help of a sterile scalpel and collected on sterile distilled water and was then filtered through a layer of sterile muslin cloth and again centrifuge. The supernatant liquid was thrown off and the suspension was then diluted with sterile distilled water to have 100 spores per low field of microscope. One healthy ear per plant was inoculated. Data were recorded after 40-45 days of inoculation at harvest after de-husking the cob and the amount of disease in each variety were expressed by a disease index formulated by Ullstrup (1949) with slight modification in the following formula :

$$\frac{n C_0 + n C_1 + n C_2 + n C_3 + n C_4 + n C_5}{N}$$

Where n=number of ears in each class. C₀, C₁, C₂, C₃, C₄, C₅=healthy, one fifth rotted, two fifth rotted, three fifth rotted, four fifth rotted and completely rotted ears. N=Total number of ears in each variety.

RESULTS AND DISCUSSION

Inoculated ears were kept under close observation to note the appearance of bluish-black perithecia, the most diagnostic feature of *Gibberella zeae* infection. In all the three methods of inoculation, perithecia formation started within a

TABLE 1. Data on disease indices in *Gibberella* ear rot with three different types of artificial ear inoculation

Sl. No.	Variety	Silk	Silk plus husk	Oat kernel
1.	Diara	3.00	4.12	4.00
2.	Amarillo-de-suba	3.31	2.71	2.60
3.	D 744	3.00	3.25	3.37
4.	Safeda	1.00	1.00	1.44
5.	D 745	0.60	1.00	1.09
6.	Ganga 5	3.00	3.50	3.05
7.	Vijay	2.50	3.00	2.33
8.	Kisan	0.25	0.35	0.27
9.	Hunius	1.00	1.25	1.10
	Total	17.66	20.18	19.25
	Mean value	1.96	2.24	2.13

fortnight of inoculation. In susceptible variety like Diara, the rate of disease development was very rapid in all the three methods, but in silk plus husk inoculation, almost the whole cob was found to be rotted and covered with abundant perithecia at 19th days of inoculation. However in oat kernel method, damage to the same extent could be found at 26th days of inoculation. The mean value of disease indices are presented in Table 1.

To find out the relationship between different methods of ear inoculation, correlation co-efficient were worked out among the disease indices of different technique of ear inoculation and the values of which are presented in Table 2.

TABLE 2. Values of co-rrrelation co-efficient among different methods of inoculation

	Factors	d. f.	'r' value
On the basis of disease index	a) Silk V. S. Silk plus husk	7	0.9416**
	b) Silk V. S. Oat kernel	7	0.9236**
	c) Silk plus husk V. S. Oat kernel	7	0.9747**

** Significant at 1% level of significance.

From the values of co-rrrelation co-efficient among the different techniques of inoculation, it is evident that there is a good correlations among different methods. Although highly significant 'r' value was obtained between silk plus husk and oat kernel inoculation, the mean value of disease index indicated that silk plus husk inoculation yielded best result.

Advantage and limitation of different techniques :

In silk plus husk inoculation complete disease expression can be observed within a short period. It takes about a fortnight for complete disease expression in susceptible variety i. e. about ten days less time is required than other methods of ear inoculation. Both in silk and silk plus husk inoculations, chance of foreign contamination is low as the inoculated areas are enclosed with coverings for a period of one week. Both the methods are suitable for high rainfall areas where spraying of inoculum over the ears are likely to be washed out by rain. Moreover use of plastic bag for covering act as an incubation chamber due to increase of humidity in the plastic bag which is also highly conducive for an early infection and quicker disease expression than to oat kernel method. On the contrary, oat kernel method is very simple and is more suitable where large number of plants need to be inoculated. There is also a less chance of washing of inoculum through rain-splash as the fungal body (mycelium and spore) tightly adhere to the surface of oat kernel.

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