Agri. Res. J. *Kerala, 1972, 10* (2): *125—127*

A TECHNIQUE FOR THE SEPARATION OF THE WHOLE EMBRYO OF RICE GRAINS FOR THE DETECTION OF FUNGAL MYCELIUM

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While studying the seed-borne nature of the stack-burn disease of rice caused by *Trichoconis padwickii* Ganguly, it was felt that the presence of the mycelium of the fungus in the embryo could be detected more conveniently if a suitable technique is evolved for separation, processing and staining of the whole embryo. The embryos of wheat and barley have been successfully separated and studied for the presence of loose smut mycelium by earlier workers like Skovortzoff (1937) Simonds (1946), Russel (1950), Russel and Popp (1951), Popp (1951 and 1958), Mortan (1960 and 1961) and Kavanagh and Mumford (1960). Since no such method was available for the separation of whole embryo of rice grains, an attempt was made to develop a suitable technique based on those evolved by Popp (1958) and Mortan (1960 and 1961)

Materials and methods

Separation of the embryo

Fully formed rice grains were collected from diseased earheads and the glumes were carefully removed by hand. The kernels were boiled for one hour in an aqueous solution containing 10% sodium hydroxide and 14% commercial liquid glass (sodium silicate) to which was added a few drops of a detergent. Approximately 600 ml. of the solution was used for 250 kernels. The boiling solution was occasionally stirred and the volume was maintained constant by periodical addition of hot distilled water. The embryos got detached from the kernels within one hour. The solution was gently stirred and liquid glass was again added till the embryos floated. The floated embryos were skimmed off for further processing. The separated embryos were washed twice in hot distilled water and then centrifuged in a 50% solution of sodium silicate for 5 minutes at 4500 r. p.m. as was done by Mortan (1960) to remove all adhering particles. After this process the embryos were again washed in distilled water.

Bleaching and cleaning

After washing, the embryos were transferred to an aqueous solution containing 25% hydrochloric acid and 5% potassium chlorate (Ainsworth

and Sampson, 1950). The embryos were kept in the above bleaching solution for two hours. They were then removed and washed thoroughly in several changes of distilled water.

The bleached embryos were first autoclaved at 5 lbs. pressure for one hour in an aqueous solution containing 15% sodium hydroxide and 12% ethanol, after which they were thoroughly washed in several changes of hot distilled water for about half an hour. The embryos were again autoclaved at 5 lbs. pressure for one to two minutes in a 3:1 mixture of ethanol and glacial acetic acid. They were then trasferred to 45% lactic acid and autoclaved at 5 lbs. pressure for one minute.

Staining

The cleared embryos were stained by autoclaving them for 15 minutes at 10 Ibs. pressure in an aqueous solution containing 45% lactic acid and 0.1% trypan blue. The embryos were again autoclaved in 45% lactic acid for one minute at 5 Ibs. pressure to remove the excess stain

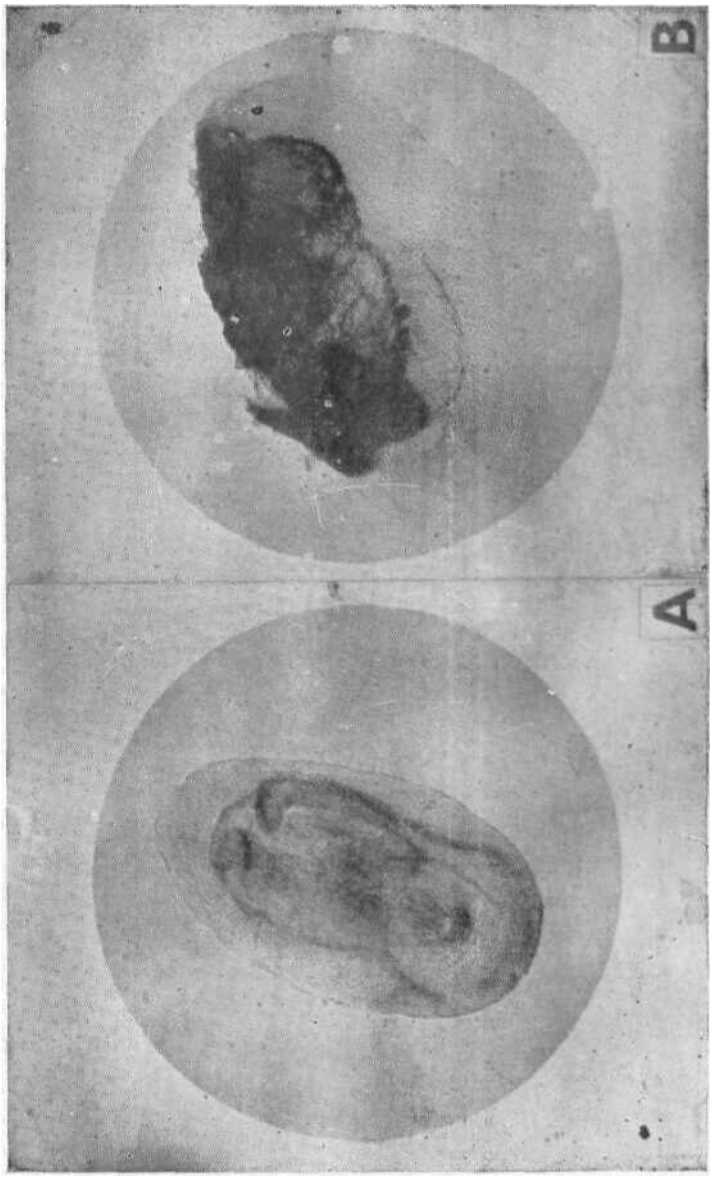
The stained embryos were arranged on 3" X 1" microscope slides in rows with the help of a zero point camel hair brush and mounted in 45% lactic acid. The infected embryos could easily be detected even under the steriomicroscope on account of the deep staining. The non-infected embryos were only lightly stained. The fungal mycelium was clearly visible under the high power objective of the ordinary light microscope (Fig. 1)

Results and Discussion

The detection of the fungus mycelium in the whole embryos of rice grains is comparatively a new line of work which has been attempted so far only on wheat and barley, chiefly by Popp (1951, 1958 & 1959) and Mortan (1960 & 1961). The technique which was developed in the present work is mainly based on the works of the above authors. Popp (1958) used 5% NaOH for the extraction of embryos of wheat and barley. But at this concentration the rice embryos failed to separate. Complete separation of embryos could be obtained only when 15% NaOH was used. However, at this concentration the endosperm and bran tissues got disintegrated.

The bleaching process used in the present work helped to increase the transparency of the embryos. For staining the embryos, aniline blue and methyl blue were also tried but they were found unsuitable. The procedure described by Mortan (1960 & 1961) were also found unsuitable. Even with trypan blue, the fungus mycelium took a deep stain only when subjected to autoclaving for 15 minutes at 10 Ibs. pressure.

FUNGAL MYCELIUM IN RICE EMBRYO



Summary

A new technique has been evolved for the detection of fungul mycelium in the whole embryo of rice grains. The embryos of infected grains were separated by boiling in 15% NaOH and bleached in an aqueous solution containing 25% HCL and 5% potasium chlorate. They were then cleared by autoclaving at 5 Ibs. pressure first in a solution containing 15% NaOH and 12% ethanol for one hour and then in a 3:1 mixture of ethanol and glacial acetic acid for 1 to 2 minutes and finally in 45% lactic acid for one minute. The cleared embryos were stained first by autoclaving for 15 minutes at 10 lbs. pressure in an aqueous solution containing 45% lactic acid and 0.1% trypan blue and then by autoclaving in 45% lactic acid for one minute at 5 lbs. pressure. The fungal mycelium was clearly visible when the treated embryos were observed under the microscope.

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