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DETECTION OF VIRUS INFECTION IN COWPEA SEEDS THROUGH EMBRYO CULTURE TECHNIQUE

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Summary

The viruses of cowpea, one rod shaped and the other spherical shaped, were chosen for the present study. The site for rod shaped and spherical shaped viruses is found to be testa and embryo respectively. Embryo culture technique for detection of virus infection in cowpea seeds has been found useful only with spherical-shaped but not with rod-shaped virus.

Introduction

Studies on seed transmitted cowpea viruses are attracting virologists' attention for the last few years. These viruses are distributed in different parts of the seeds. The two important viruses reported from India, one rod shaped (Sharma & Varma, 1975) and the other spherical shaped (Chenulu, Sachchidananda & Mehta, 1968) were studied to determine the seed borne nature of these viruses. During the studies, embryo culture technique was employed in order to investigate the mode of transmission and detect the virus infection in the cowpea embryos.

Experimental

Seeds of cowpea (*Vigna sinensis* Savi) were collected from healthy as well as rod-shaped and spherical-shaped virus infected plants. For embryo culture study, one hundred seeds (each healthy and diseased) were wetted with 95 per cent alcohol, soaked overnight in sterile water and then passed through 0.1 per cent mercuric chloride and 30 per cent hydrogen peroxide for 1 min each, respectively. They were thoroughly washed in sterile water. After surface sterilization, the seeds were aseptically dissected, by first removing the testa. The embryos were carefully taken out by means of a flamed sharp scalpel and planted on various media in culture tubes. The tubes were kept at temperatures ranging from 25-27°C under continuous illumination of 80 foot candles. Composition of different media are given in Table 1. For detecting the presence of virus infection in the embryos and other parts of

TABLE 1

Composition of media used for cultivating cowpea embryos

Chemicals	*Media*				
	A	*B*	*C*	*D*	*E*
Napthalene acetic acid (NAA) (mg)	-	-	0.1	0.1	-
Indole acetic acid (IAA) (mg)	-	-	-	-	2.0
Biotin (mg)	-	-	0.1	0.1	-
Calcium pentathenate (mg)	-	-	0.1	0.04	-
Casein hydrolysate (mg)	-	200	-	200	200
Agar (mg)	8	8	8	8	8
Murashige & Skoog's medium (1962) (ml)	1000	1000	1000	1000	1000

the seeds, *Chenopodium amaranticolor* Coste & Reyn were mechanically inoculated using carborudum.

Separate lots of diseased and healthy seeds were sown in vermiculite and watered daily. Germination started in 7-15 days. To observe embryo germination, excised embryos of diseased and healthy seeds were put in the culture tubes containing medium D.

In addition, embryos were excised from both types of diseased and healthy seeds and transplanted aseptically on different media A, B, C, D, and E.

Results

Rod-shaped virus produced local lesions in 3 days and spherical shaped virus within 10 days.

Site of seed infection

In the case of rod-shaped virus, seed coats produced local lesions on *C. amaranticolor* except one or two cases out of 50 seeds, cotyledons and embryos were free from the virus. The seed coats were totally free from the virus.

Loss of seed viability

The embryos germinated within 3-5 days. The germination percentage of seeds and embryos are given in Table 2. Embryos germinated better when sown on medium D.

Recovery of seed viability through embryo culture

It was observed (Table 1) that healthy embryos showed very slow growth in media D and E. In the other media, they did not grow. In the case of rod-shaped virus in all media the embryos grew into normal plants except in medium B where the embryos produced profuse callus with feeble leaves and fewer roots. In the case of spherical shaped virus, growth was excellent in medium D. However, medium A produced normal growth while media C and E plants were comparatively stunted with narrow leaves. In medium B, leaves were pale yellow and small and roots were few. Different media induced callus formation and rooting of embryos infected with two types of viruses. Healthy embryos on media D and E on germination produced rather poorly developed roots, stems and leaves that grew very slowly (Table 3).

TABLE 2

Germination percentage of seeds and excised embryos from healthy and diseased seeds

Seed types	*Germination in soil*	*Germination of embryos in medium D*
Healthy	67	85
Infected with rod-shaped virus	55	65
Infected with spherical-shaped virus	63	76

TABLE 3

Growth patterns of cowpea embryos from diseased seeds in different media

Type	*Medium*	*Growth pattern*	*Germination %*
Rod	A	PR SS NL	100
	B	PC PR FL	25
	C	PC MR SS NL	100
	D	PC PR SS NL	20
	E	FR SS NL	20
Spherical	A	PR SS NL with mosaic	10
	B	FR shs pale yellow leaves	30
	C	PC PR SS NL	30
	D	PR as NL	40
	E	PR shs NL	80

Embryos showing organ formation are considered as germinated, PR = profused rooting , FR = few roots, MR = medium rooting, SS = slender shoot, shs = short shoot, PC = profuse callusing, NL = normal leaf, FL = feeble leaf, NL = narrow leaf.

Discussion

There was no improvement in germination of diseased embryos (in the case of rod-shaped virus) with increase in nutrient components as observed by Mishra, Raychandhuri & Jagadish.

(1967) in runner bean. There was no correlation between the germination percentage and growth pattern. So recovery of seed viability through embryo culture in rod-shaped virus infected cowpea seeds was not possible in this case. In case of spherical-shaped virus, seed viability was improved by embryo culture where the site of infection was embryo. Growth was organized.

In the present study, the embryo culture technique has been found useful for detection of virus infection in cowpea seed lots. The technique can enhance germination of spherical-shaped virus infected cowpea seeds but not those infected by rod-shaped virus. Mishra *et al.* (1967) were able to get successful results with runner bean mosaic virus. Crowley (1957 a, b) observed that developing tomato embryos did not inactivate the presence of virus in the medium on which embryos were grown. Bean mosaic virus which is seed transmitted was found to infect embryos but no evidence of virus inactivators was found in the developing embryos.

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