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The infectibility of cowpea mesophyll cells by tobacco necrosis virus

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Abstract

The time required for infectious tobacco necrosis virus (TNV) to pass through the epidermis of cowpea leaves after mechanical inoculation depended on plant age and environmental temperature. At 22°C the passage time was about 2 h, but at 32°C it was only 10 min. Water stress seemed to play a role in the transport of infectious virus into the mesophyll. It was possible to infect cowpea mesophyll cells with TNV directly by using a fine brush without carborundum.

Differences between tobacco and cowpea in the establishment of virus infection are discussed.

Additional keyword: epidermis.

Introduction

The ability of a virus to induce disease in an infected plant is the result of a complex interaction of many factors determined by the biological, biochemical and genetic characteristics of the virus on the one hand and the reactivity of the host on the other hand. The processes involved in the infection of plants by viruses are still obscure. Resistance to infection may be controlled by a single gene of the host or by many genes. One kind of resistance mechanism in a plant to virus infection is the hypersensitive reaction. After inoculation of hypersensitive leaves with a virus, necrotic local lesions develop. The hypersensitive reaction can be interrupted by removal of the epidermis soon after inoculation (Shimomura, 1977).

Injury of the epidermal cells is a prerequisite for successful mechanical inoculation with viruses. Few data are available on the changes that occur inside epidermal cells after wounding, although injury to the surface of epidermal cells has been studied (Herridge and Schlegel, 1962; Brants 1964, 1965). Favali et al. (1977) examined the early modifications in the epidermal cells of cowpea and *Nicotiana glutinosa* leaves after mechanical sham inoculation. Alternations of the cell wall were no longer visible 6 h after inoculation, while cytoplasmic vacuolation persisted.

Bailiss and Plaza-Morales (1980) suggested that post-inoculation water stress may affect the possible establishment of infection by a mechanically transmitted virus in a local lesion host. A rapid, but transient post-inoculation reduction in leaf water potential resulted in increased lesion production when *Phaseolus vulgaris* was infected with tobacco necrosis virus (TNV). Water stress only affected the early stages of the infection process. They assumed that water stress affected the receptivity of infectible sites.

The leaf epidermis is involved in the formation of necrotic lesions (Dijkstra, 1962; Coutts, 1980; Wieringa-Brants, 1981). The transport of infectious virus material through the epidermis into the mesophyll occurred more rapidly in plants darkened 24 h before inoculation than in control plants. It seems likely that cytoplasmic streaming plays an important rôle in transportation.

The object of this study was to determine the minimal time of contact between epidermis and mesophyll needed to induce local lesions and to enhance the infectibility of mesophyll cells in cowpea leaves after inoculation with TNV.

Materials and methods

Plants. Cowpea plants *(Vigna unguiculata* L. Walp., cv. Blackeye Early Ramshorn) were grown and treated as described earlier (Wieringa-Brants, 1981).

Virus. Pressed sap of cowpea leaves infected with TNV strain A or D served as inoculum and was diluted to elicit about 100 lesions on a cowpea half-leaf. TNV strains were purified on a sucrose gradient or according to Kassanis (1964). Primary cowpea leaves at 22°C were inoculated on the abaxial surface with carborundum as an abrasive. At various times after inoculation the leaves were stripped, i.e. their lower epidermis was removed with a fine forceps. Local lesions were counted on the stripped and non stripped parts, three days after inoculation.

Results

Plant age. The influence of the age of cowpea seedlings on the minimal period of contact between epidermis and mesophyll after virus inculation to induce lesion formation was determined (Table 1). In primary leaves of 10-day-old cowpea seedlings a contact period of at least 2 h was required, whereas in 20-day-old seedlings this period was at least 4 h. The same inoculum produced more lesions in the younger leaves.

When 10-day-old seedlings had been darkened 24 h before inoculation the minimal contact period was 50 min (data not shown). Therefore markedly decreased the passage time of infectious virus material. The same results were obtained with TNV-D.

Water stress and high temperature of 32 °C. The possible effect of post-inoculation leaf water balance on the period required for virus to pass through the epidermis was examined. Post-inoculation water deficit was achieved by a high temperature treatment of 32°C. Cowpea leaves, inoculated with TNV, were immediately transferred to 32°C on dry filter paper under continuous fluorescent light (6000 lx) for different periods. Thereafter, half of each leaf was stripped and the leaf was placed at 22 ± 3°C on wet paper to evoke lesion formation. Results (Table 2) show that dry conditions did increase the total number of lessions in both non-stripped and stripped non-darkened half-leaves. At 32°C, a remarkably short 10 min interval between inoculation and stripping was sufficient to induce lesions in the mesophyll. In another experiment leaves were kept in darkness 24 h before inoculation with 1 : 1 diluted inoculum. Similar results were obtained (Table 2).

Table 1. Effect of removal of the lower epidermis after inoculation with TNV strain A on lesion formation in cowpea leaves of different ages.

Period of contact between epidermis and mesophyll (h)	Lesion numbers[1)]			
	10-day-old seedlings		20-day-old seedlings	
	epidermis		epidermis	
	not removed	removed	not removed	removed
0.5	153	0	18	0
1	130	0	25	0
2	155	0	17	0
3	140	6	28	0
4	127	9	31	0
5	197	91	21	10

[1] Mean values from 6 replicate half-leaf comparisons for each time interval

Tabel 1. Invloed van de verwijdering van de onderepidermis na inoculatie met TNV stam A op de vorming van lokale lesies in cowpea-bladeren van verschillende leeftijden.

Tabel 2. Influence of desiccation and high temperature (32°C) on the passage time of TNV through the epidermis of cowpea leaves.

Light condition[1]	Time (min) at 32°C between inoculation and stripping	Lesion numbers[3]	
		epidermis	
		not removed	removed
–	0[2]	462	0
–	10	660	10
–	20	820	17
–	30	1013	16
–	40	1090	33
–	50	950	31
+	0[2]	225	14
+	10	376	20
+	20	458	30
+	30	420	20
+	40	390	15

[1] –, leaves not darkened; +, leaves kept in darkness 24 h before inoculation
[2] Control kept at 22°C for 70 min between inoculation and stripping
[3] Mean values from 10 replicates

Tabel 2. Invloed van uitdroging en hoge temperatuur op de passagetijd van TNV door de epidermis van cowpea-bladeren.

The passage time of virus through the epidermis at 32°C was also determined in other host-virus combinations. *Chenopodium quinoa* L. with TNV-A and *Phaseolus vulgaris* L. cv. Dubbele Witte zonder draad with TNV-A were used in similar experiments. In all cases a 10 min interval between inoculation and stripping was sufficient to induce lesions in the mesophyll.

No difference in lesion number (Table 2) was noted when the interval was increased from 10 to 30 min. This would suggest that the passage of infectious virus material through the epidermis at 32°C is momentary and not dependent on virus multiplication inside the epidermal cells. Then it should be possible to infect cowpea mesophyll cells directly with virus.

Inoculation of mesophyll tissue. Direct inoculation of mesophyll with TNV was unsuccesful in former experiments (Wieringa-Brants, 1981). Mesophyll inoculated with carborundum as an abrasive was badly injured. Other methods of infecting the mesophyll tissue were tried. Carborundum (500 mesh) was used in comparison with fine carborundum (100 mesh) in infection tests on cowpea mesophyll with TNV-A and D. With fine carborundum as an abrasive a few lesions were formed in the mesophyll. When TNV in pressed sap or in purified preparation was applied to the mesophyll with a fine brush without using carborundum, more lesions appeared. Many lesions were formed in 9-12-day-old cowpea seedlings grown at 22 ± 3°C when the leaves were kept on dry filter paper for 15 min to 1 h after picking, depending on the environmental conditions. The lower epidermis was then removed and the mesophyll was inoculated with TNV with a fine brush. TNV inoculum applied to the non-stripped half-leaf by rubbing with carborundum resulted in as many lesions (100 average) as in the stripped half, inoculated with the same inoculum applied with a fine brush without carborundum.

Discussion

The time required for TNV to pass through the epidermis of cowpea leaves was dependent on plant age and environmental temperature. When cowpea leaves were kept at 32°C immediately after inoculation with TNV, the virus passed through the epidermis in only 10 min. This fact supports Bailiss and Plaza-Morales (1980) who suggested that post-inoculation water stress influences the likelihood of infection, but only in the early stages.

Jedlinski (1956) noticed that virus infection is related to the interval between wounding and inoculation. This author reported that in cowpea leaves, the susceptibility for TNV increased for an interval of up to 10 min between wounding and inoculation and then decreased. The interval had little or no effect on infection of *N. tabacum* species by TNV. It is possible that build up of water stress is responsible for the period of optimum receptivity of the host to virus after wounding. The processes are host-specific since the interval had no effect on TNV infection of *N. tabacum* species. It is not known whether the condition of stomata is important for virus entry and transportation.

Beans are more susceptible if kept at high temperature of 30°C before they are inoculated with TNV (Harrison, 1956). McCarthy et al. (1976) stated that in the bean the rate of TNV accumulation seems to be a result of the opposing effects of

temperature on the number of infected cells and virus multiplication within them in an early stage of infection.

Coutts (1980) and Wieringa-Brants (1981) have suggested that the epidermis has an active rôle in the infection process of cowpea leaves by TNV. An interaction between epidermis and mesophyll seems necessary to induce local lesions in hypersensitive leaves. When exposed mesophyll tissue is inoculated, the epidermis of the opposite side is still present and may influence lesion formation.

Coutts (1980) could not detect infectious TNV in epidermal cowpea strips up to 2 days after inoculation. Examination of viral activity in isolated epidermal strips of cowpea 4 days after infection with TNV (Wieringa-Brants, 1981) revealed that multiplication in the epidermis had occurred, which could not be attributed to the few mesophyll cells still attached to the epidermis. Attempts to infect exposed cowpea mesophyll with purified TNV droplets or by injection into the intercellular spaces below the epidermis failed to produce lesions (Coutts, 1980). The infection of cowpea mesophyll tissue with TNV was obtained in this study when a fine brush was used on leaves under water stress. It seems that the method of inoculation and the environmental conditions make the difference in establishing virus infection.

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Samenvatting

De infecteerbaarheid van cowpea-mesofylcellen door tabaksnecrosevirus

De mogelijkheden om cowpea-mesofylcellen met TNV te infecteren, hetzij via de epidermis, hetzij rechtstreeks, werden onderzocht. De minimum tijd die infectieus virusmateriaal nodig heeft om de epidermis te passeren werd bepaald. De ouderdom van de zaailingen speelde daarbij een rol. Bij 20 dagen oude planten bedroeg de passagetijd meer dan 4 uur, in 10 dagen oude planten was meer dan 2 uur nodig voor passage. De temperatuur van de omgeving was een belangrijke factor. In cowpea-bladeren, direct na inoculatie met TNV bij 32°C gezet, bleek de passagetijd slechts 10 minuten te zijn. Verduistering van de planten 24 uur voor inoculatie bekortte de passagetijd aanzienlijk. Ook speelde water-stress in het blad door hoge temperatuur na inoculatie een rol in de vroege infectiestadia. Het was mogelijk, cowpea-mesofyl direct te infecteren, indien cowpea-bladeren gebruikt werden van 9-12 dagen oude zaailingen die opgekweekt waren bij 22 ± 3°C. De bladeren werden gedurende ± 1 uur na afplukken op droog filtreerpapier gelegd, daarna werd de onderepidermis met een fijn pincet verwijderd. Inoculatie van het naakte mesofyl met TNV onmiddellijk na het strippen met een fijne penseel zonder carborundum resulteerde in eenzelfde aantal lesies als in de niet-gestripte helft, die geïnoculeerd was door wrijven met hetzelfde TNV-inoculum in aanwezigheid van carborundum.

Het schijnt dat de processen, die betrokken zijn bij het tot stand komen van een TNV-infectie in cowpea en tabak, van elkaar verschillen.

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