Acta Bot. Croat. *41*, 1—5, 1982.

CODEN: ABCRA2
YU ISSN 0365—0588

UDC 582.739:576.858.8=20

INFLUENCE OF DARKENING AND MODE OF INOCULUM PREPARATION ON PEA SUSCEPTIBILITY TO CUCUMBER MOSAIC VIRUS

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Received December 16, 1981

The purpose of these glasshouse investigations was to find methods which can increase the susceptibility of pea to infection with cucumber mosaic virus. Inoculum prepared with phosphate buffer (pH 7.0) proved to be most effective because 92% of inoculated plants were infected. When only tap water was added to the plant material before grinding, the effectiveness was 58%. Adding water after the grinding of plant material was less favourable (31%). Darkening of plants for one or two days before inoculation reduced a great deal of infectivity (31% and 23% respectively). Addition of plant saps of pea or blue lupine diminished very much the infectivity so that only 12 and 16% plants were infected, respectively.

Introduction

During research into legume viroses, especially those of pea, we were faced with serious difficulties connected with mechanical infection by cucumber mosaic virus (CMV). Usually it appeared to be a local and not a systemic infection. This made it extremely difficult to determine the harmfulness of this virus or to test plant breeding materials. The purpose of this work was to increase the pea susceptibility to CMV by means of plant darkening before inoculation and also by changing the methods of inoculum preparation.

Material and Methods

Pisum sativum cv. Flavanda plants and CMV isolated from *P. sativum* type *aphylla* ("moustache") were used in this experiment. The

virus was maintained on tobacco cv. Turkish and on pea. Leaves of Turkish tobacco showing symptoms of CMV infection were also used for inoculum preparation.

The experiment was carried out in a glasshouse from March 17 to May 22, 1979. Average temperature was about 16° C and the day length 12 to 16 h. Until inoculation the plants had been exposed to mercury light (LRFR-1) of 250 W power from a distance of 1 m for 7 h a day.

Pea plants were grown in ceramic pots of 15 cm diameter filled with steamed soil. Plants watering was controlled.

In order to investigate the influence of darkening on pea susceptibility to CMV, the pea plants were darkened one and two days before inoculation. The second group of trials was related to the influence of the mode of preparing inocula. It was investigated whether it was better to add water before or after grinding the plant material. In other trials the influence of phosphate buffer pH 7 and plant saps of blue lupine and pea was tested. These solutions were separately added to the pea material before grinding (Tab. 1). The same ratio 1 : 1 of green matter to diluent volume was preserved in all combinations.

Plants were inoculated in the stage of 3—4 leaves. In all cases 3 bottom leaves were inoculated by using carborundum powder. After inoculation the plants were washed with water.

Many observations were carried out, and plants with systemic infection were marked each time. Results of all trials were elaborated statistically.

Results

Influence of plant shading on inoculation effectiveness

It turned out, quite unexpectedly, that darkening did not affect favourably the inoculation effectiveness (Tab. 1). On the average, inoculation effectiveness of darkened plants was 50% lower than that of non-darkened plants, and darkening for 2 days was slightly more harmful than darkening for one day. Only 24 % of 2 day darkened plants and 31 % of one day darkened became infected, while up to 58.3 % of control plants showed infection symptoms.

Influence of inoculum preparation on inoculation effectiveness

It was observed that the moment at which water was added to the inoculum had a significant influence on the inoculation effectiveness. Grinding the infected plant material with water proved to be significantly better than adding water after grinding (Tab. 1). In the first case 58.3 % of inoculated plants were infected while in the second case only 30.6 %.

Best results were obtained with inoculum prepared with phosphate buffer (Tab. 1). Out of 49 inoculated plants 45 underwent systemic infection, i. e. 91.8 %. Pea and blue lupine saps used for inoculum preparation turned to be rather unfavourable. Only 12.2 and 16.3% of inoculated plants were infected after application of these inocula. Control experiment was performed by adding tap water instead of plant sap. The difference between plant sap and control experiments with water was highly significant.

Table 1. Dependence of pea susceptibility to cucumber mosaic virus upon the plant treatment and mode of inoculum preparation

Plant treatment and mode of inoculum preparation	Number of plants showing disease symptoms on 10 day after inoculation	13	17	21	25	Percentage of infected plants at the end of experiment
2 days darkening Inoc. with water	4	5	8	12	12	24
1 day darkening Inoc. with water	1	7	13	14	15	31.3
No darkening Inoc. with water	12	18	24	28	28	58.3
Water was added after the grinding	6	11	14	15	15	30.6
Inoculation with pea sap	0	3	4	6	6	12.2
Inoculation with blue lupine sap	5	6	7	8	8	16.3
Inoculation with phosphate buffer	18	38	44	45	45	91.8

Note: 48 to 50 plants were inculated in each trial.

Table 2. Effect of the length of incubation on the growth of pea plants infected with cucumber mosaic virus

	Length of incubation in days				Healthy plants
	10	13	17	21	
Number of plants in experiment	46	42	27	14	49
Mean height of plants in cm	10.4	11.4	14.2	16.3	36.5
Relative values in %	29	31.5	39.3	45	100

Additionally, the height of plants was measured 21 days after inoculation. As expected, earlier appearance of symptoms caused more severe height checking (Tab. 2, Fig. 1). Plants showing first symptoms of infection on the 10th day after inoculation attained only 29 % of the height of healthy plants 21 days after inoculation. However, plants showing the first symptoms of infection on the 21st day after inoculation reached 45 % of the height of healthy plants. This shows that the virosis has a harmful effect on plants right after the infection and before the appearance of disease symptoms.

Discussion

Darkening of plants before inoculation used commonly in virology in order to increase their susceptibility to virus infection turned out to be a step decreasing the effectiveness of pea inoculation by CMV. In other words, it decreased plant susceptibility to infection. However, in other cases darkening can be propitious for inoculation of plants. According to Błaszczak (1963) 76 % and 85 % of yellow lupine plants were infected with bean yellow mosaic virus when they were darkened for 3 days. In control experiments with non darkened plants the respective values were 3 and 31 %.

Good infectivity was shown when the inoculum was prepared with water. It turned out that grinding the plant material with water produced an inoculum of considerably higher infectivity than in the case when water was added after the grinding of plant material. A similar procedure gave good results also in the case when red clover was infected with bean yellow mosaic virus (Błaszczak and Kowalska 1974).

It is also commonly known that buffers used for inoculum preparation have a favourable effect on infectivity of inoculum (Błaszczak and Kowalska 1974, Matthews 1970). In our experiment this procedure gave best results (92 % of infected plants). It is very probable that such a high efficiency of pea inoculation is not easy to reach again. There are other factors which can influence the effectiveness of inoculation (Matthews 1970).

Inocula prepared with pea and blue lupine sap proved to be least infective. It can be assumed that inhibitors present in sap can considerably reduce the infectivity of CMV. Francki (1964) showed that the sap from cucumber leaves added to purified CMV decreased its infectivity hundredfold.

References

Błaszczak, W., 1963: Badania nad waskolistnością łubinu zółtego w warunkach Polski Zachodniej (Studies on narrow-leavedness of yellow lupin in West Poland). Roczniki Wyzszej Szkoły Roln. Poznań, XV.

Błaszczak, W., Cz. Kowalska, 1974: Efektywność izolacji i przenoszenia trzech wirusów roślin motylkowatych na drodze mechanicznej (Efficiency of isolation and mechanical transmission of three papilionaceous plant viruses). Zesz. Probl. Post. nauk rol. 156, 121—129.

Francki, R. I. B., 1964: Inhibition of cucumber mosaic virus infectivity by leaf extracts. Virology 24, 193—199.

Matthews, R. E. F., 1970: Plant Virology. Academic Press, New York and London.

Fig. 1. Plant 1 is healthy; on plant 2 systemic infection appeared 17 days after inoculation, and on plant 3 it appeared 13 days after inoculation.

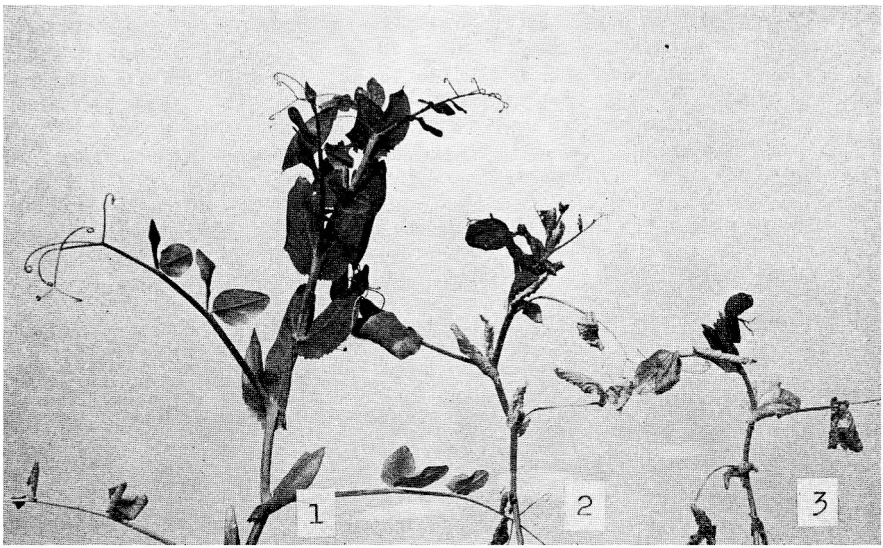

Fig. 1.

SAŽETAK

UTJECAJ ZAMRAČIVANJA BILJAKA I NAČINA PRIREĐIVANJA INOKULUMA NA PRIJEMLJIVOST GRAŠKA ZA VIRUS MOZAIKA KRASTAVCA

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Cilj istraživanja, koja su obavljena u stakleniku, bio je da se pronađe metoda koja će povećati prijemljivost graška za infekciju virusom mozaika krastavca. Ustanovilo se da je inokulum priređen s fosfatnim puferom pH 7 bio najefikasniji, jer je oboljelo 92 % inokuliranih biljaka. Ako se vodovodna voda dodavala biljkama prije gnječenja, efikasnost je bila 58%. Dodavanje vode poslije gnječenja bilo je manje povoljno (31 %). Zamračivanje biljaka u vremenu od jednoga ili dva dana prije inokulacije znatno je smanjivalo infektivnost inokuluma, tako da je efikasnost iznosila samo 31 %, odnosno 23 %. Dodatak biljnog soka od graška ili modre vučike reducirao je znatno infektivnost, tako da je oboljelo samo 12 %, odnosno 16 % biljaka.

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