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Development of Mild Strains of *Papaya Ringspot Virus*-Papaya Strain

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ABSTRACT

An attempt was made with BARI papaya-1 to develop the mild strains of Papaya ringspot virus-papaya strain (PRSV-P) to use in cross protection. Three mutagents (viz. nitrous acid, sodium azide and UV radiation) with different concentrations and exposure time were used in this experiment. Mutagenic treatments of extraction of PRSV-P infected sap with 0.075M nitrous acid, 2% sodium azide, and exposure to UV radiation for 1 and 2 minutes were observed to be the best in the production of effective mild strains of PRSV-P. The mild strains of PRSV-P containing papaya seedlings were faced to challenge inoculation test. It was observed that younger seedling condition delayed the virus infection as compared to older ones.

Key words: PRSV-P, mutagenic treatments, cross protection.

INTRODUCTION

Papaya (*Carica papaya*) is an important fruit of tropical and subtropical regions of the world. It is especially discernible due to its high nutritive value, taste and diversified uses as vegeTable, fruit and preparation of various food items and in confectioneries. The papaya fruit an abundant source of carotene, the precursor of vitamin A (Tee *et al.* 1988). It contains reasonable amount of vitamin C, riboflavin, niacin and a good source of protein, fat, carbohydrate, calcium, phosphorus and iron (Bosh, 1985). The industrial use of papaya includes the production and preparation of papain, pectin and carpaine alkaloids (Yon, 1994).

Papaya production is greatly hampered due to some diseases of which, a viral disease caused by *Papaya ringspot virus*-papaya strain (PRSV-P) is considered to be the most important and severe one (Wang *et al.* 1978). The virus infects the plant at any stage of growth producing different types of symptoms like, mosaic, vein-clearing, chlorotic spots, fern leaf, shoe-string, distorted fruits, stunted growth etc (Husain and Verma, 1994; Dahal *et al.* 1997). The disease caused by severe strains of PRSV-P can be avoided if the plants are first inoculated with mild strains of the same virus and the principle is popularly known as cross protection (McKinney 1929). The development and selection of mild strains of PRSV-P has been successfully done and used for cross protection by Yeh *et al*. (1988). The cross protection technique can be achieved by developing the mild strains of PRSV-P through mutagenic treatments. Therefore, the present study

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was undertaken to develop mild/avirulent strains of PRSV-P through mutagenic treatments and to test their effectiveness.

MATERIALS AND METHODS

The research was conducted at the research farm as well as laboratory of Bangabandhu Sheikh Mujibur Raman Agricultural University, Gazipur during March 2002 to December 2002 as a part of thesis work. Seedlings of papaya variety 'Shahi' (BARI papaya1) was raised in earthen pots and maintained in an insect proof net house. Three mutagents (viz. nitrous acid, sodium azide and UV radiation) were used for developing the mild strains of PRSV-P. Nitrous acid was prepared according to the procedures followed by Yeh and Gonsalves (1984). Different concentrations of 50 ml sodium acetate (0.05M, 0.075M, 0.1M and 0.125 M) were adjusted to pH 6 by adding acetic acid. The mixtures were incubated at 20C for 30 minutes. Then the reaction was stopped by adding equal volume of 0.1 M potassium phosphate buffer (p^H 7). 10% sodium azide solution was prepared from which a series of solution i.e., 5%, 4%, 3%, 2% and 1% solution were made. UV radiation available in the clean bench of microbiology lab of BSMRAU was used in the mutagenic treatment.

Severely PRSV-P infected papaya leaves were collected from the experimental field and ground in distilled water (10g/10ml). Then the macerated leaf tissues were treated with freshly prepared nitrous acid using different concentrations each for 10 minutes. The samples were immediately inoculated to 100 papaya seedlings with the four selected concentrations, where 25 seedlings were inoculated with each of the concentrations. All these activities were performed in an insect proof net house and the seedlings were managed with regular care. The inoculation was achieved by using 0.1 M potassium phosphate buffer (p^H -7) and carborandum powder. A total of 25 seedlings were inoculated with fresh sap using potassium phosphate buffer and corborandum powder, which were treated as control. Similarly, the macerated leaf tissues were treated with different concentrations of sodium azide (i.e. 5% to 1%) each for 10 minutes and inoculated to papaya seedlings. In case of UV-radiation treatments, the macerated leaf tissues were filtrated through cheese cloth and crude sap was placed in five petridishes. The petridishes were then exposed under UV radiation in the clean bench for 1 minute for 1st treatment, 2 minute for 2nd treatment, 3 minute for 3rd treatment, 4 minute for 4th treatment, 5 minute for 5th treatment. The samples were then immediately inoculated to papaya seedling following the previous procedures. In every case 75 days old papaya seedling were used for inoculation.

The papaya seedlings inoculated along with control were regularly inspected in the net house for the appearance of symptoms up to 14 days. The mild and no symptoms possessing papaya seedlings were further inoculated with sever symptoms for evaluation of the mild strains.

The asymptomatic plants were inoculated with PRSV-P infected test sample every 14 days interval for three times maintaining appropriate control. The inoculation was done on the upper leaves of the papaya seedling with severely PRSV-P infected crude leaf sap. Then the seedlings were placed in the field for observation of PRSV-P symptoms in the leaves. This process is popularly known as challenge inoculation which was described by Yeh *et al.* (1988).

RESULTS AND DISCUSSION

The results of mutagenic treatments to develop mild/virulent strains of PRSV-P are summarized in Table 1, 2, and 3.The three concentrations of nitrous acid- 0.075M, 0.1M and 0.125M produced 64%, 8% and 8% asymptomatic plants and 24%, 28% and 32% plants with mild symptoms, respectively. It was also observed that 0.05M nitrous acid treatment induced 20% plants having mild symptoms but there was no asymptomatic (symptomless) plant. Higher dose of nitrous acid might increase the replication of virus particles rather than to induce mutation. The phenomenon was also observed by Yeh and Gonsalves (1984). In case of sodium azide treatment 2% and 3% conc. out of five treatments induced asymptomatic plants. In all, 60% and 36% plants having mild and without symptoms were observed in 2% sodium azide treatment while it was recorded as 12% and 8% respectively in case of 3% sodium azide treatment. Of the five UV treatments, 1, 2 and 3 minutes treatment resulted 72%, 68% and 32% plants having mild symptoms, respectively. The asymptomatic plants counted in UV treatment were 16%, 20% and 8% when 1, 2 and 3 minutes

exposure to UV radiation were applied. Besides, 4 and 5 minutes exposure time developed 36% and 20% plants with mild symptoms but none of those were counted under asymptomatic group. In all cases, control treatment i.e., plants inoculated with the extracted sap of PRSV-P infected papaya leaves without any mutagenic treatments developed 100% plants with severe symptoms having no mild or asymptomatic plants.

Table 1. Development of mild strains of PRSV-P by nitrous acid treatment

Conc. of nitrous acid (M)	% of plants showing differential reaction		
	Severe symptoms	Mild symptoms	Symptomless
0.050	80	20	0
0.075	12	24	64
0.100	64	28	8
0.125	60	32	8
Control	100	0	0

Table 2. Development of mild strains of PRSV-P by sodium azide treatment

Conc. of sodium azide (%)	% of plants showing differential reaction		
	Severe symptoms	Mild symptoms	Symptomless
1	88	12	0
2	4	60	36
3	80	12	8
4	92	8	0
5	96	4	0
Control	100	0	0

Table 3. Development of mild strains of PRSV-P by UV treatment

Exposure to UV-radiation (minute)	% of plants showing differential reaction		
	Severe symptoms	Mild symptoms	Symptomless
1	12	72	16
2	12	68	20
3	60	32	8
4	56	36	8
5	80	20	0
Control	100	0	0

Table 4. Reaction of asymptomatic papaya plants inoculated with severe symptoms of PRSV-P

Days after challenge inoculation (Symptom expression)	No. of papaya plants without severe symptoms challenged at days								
	14			28			42		
	*N	[x]S	U	N	S	U	N	S	U
0	10	10	10	10	10	10	10	10	10
7	10	10	10	10	10	10	8	6	7
14	10	10	10	10	10	7	8	5	6
21	10	10	10	10	10	6	6	5	6
28	10	10	10	10	10	6	6	5	5
35	10	10	10	10	10	6	6	5	5
42	10	10	10	8	6	6	6	5	5
49	10	10	10	8	6	6	6	5	5
56	10	10	10	8	6	6	6	5	5
63	10	10	10	8	6	6	6	5	5
70	10	10	10	8	6	6	6	5	5
77	10	10	10	8	6	6	6	5	5
84	10	10	10	8	6	6	6	5	5
91	10	10	10	8	6	6	6	5	5
98	10	10	10	8	6	6	6	5	5
105	10	10	10	8	6	6	6	5	5

*N- Nitrous acid, [x]S- Sodium azide, U- UV radiation.

The results of challenge inoculation test were summarized in Table 4 which indicates reaction of asymptomatic papaya plants developed through different mutagenic treatments. The results demonstrated that none of the plants of any three groups produced any symptomatic plants up to 105 days when challenged at 14 days (after 14 days of mutagenic treatment). When challenged at 28 days with severe strains of PRSV-P, 2 plants developed symptoms at 42 days in case of nitrous acid treatment and in sodium azide treatment 4 plants showed symptoms at 49 days and another 1 plant showed symptoms at 84 days. In case of UV radiated plants, 3 plants showed symptoms at 14 days and another one at 21 days. Again, when challenged at 42 days, 2 plants showed symptoms after 14 days of challenge inoculation, another 2 plants at 21 days and lastly another 1 at 105 days in case of nitrous acid treatment. The treatment with sodium azide resulted 4 plants with PRSV-P symptoms after 7 days of challenge inoculation and another 1 plant at 14 days. Similarly, 3 plants developed symptoms at 7 days, another one at 14 days lastly another 1 at 28 days in case of UV treatments.

The results so far obtained in the present study revealed that all the three mutagens developed mild or avirulent strains of PRSV-P although all concentrations or cexposure time were not equally effective. In case of nitrous acid, 0.075M was found to be highly effective while 2% sodium azide treatment was found to be superior in developing mild/avirulent strains of PRSV-P. In case of UV radiation 1 and 2 minute exposure were observed to be equally effective than the other exposure time. The results also suggested that with the increase of days of challenge inoculation the response of mutant plants become decreased i.e. younger plants delayed the virus infection than the older plants. Yeh and Gonsalves (1984) and Yeh *et al.* (1988) induced attenuation in PRSV-P and developed mild strains through nitrous acid mutagenic treatment as obtained in the present study. The use of other two mutagens (Sodium azide and UV radiation) to develop mild strains was demonstrated by McKinney (1929) for *Tobacco mosaic virus* (TMV) and other viruses. In the present experiment, both the mutagens (Sodium azide and UV radiation) were proved to be effective in developing mild strains of PRSV-P which supports the findings of McKinney (1929).

CONCLUSION

Mild strains of *Papaya ringspot virus*-Papaya strain (PRSV-P) can be developed successfully by treating the virus infected sap with mutagens like nitrous acid, sodium azide and UV radiation. Among these mutagens 0.075 M nitrous acid, 2% sodium azide and exposure to UV radiation for 1 and 2 minutes were observed to effective. The mild strains might be applied for using cross protection against severe strains of PRSV-P.

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