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ROLE OF REDUCING AGENTS ON ELECTROLYTE LEAKAGE FROM RICE CHLOROPLASTS INFUSED WITH *XANTHOMONAS ORYZAE* PV. *ORYZAE* DYE

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Chloroplast extract of rice cultivars resistant (IR20) and susceptible (TNI) to bacterial blight were infused with virulent cells of *Xanthomenas oryzae* pv. *oryzae* and incubated for various time intervals. Electrolyte leakage was maimum when chloroplasts of susceptible cultivars were infused with bacterium. Treatment of the extracted chloroplasts with reducing agent like sodium thio-sulphate at the time of bacterial infusion significantly reduced the intensity of electrolyte leakage from the rice chloroplasts.

Key Words : Electrolyte leakage, Chloroplast, *Xanthomenas oryzae*

Bacterial blight (BB) is a serious disease in most of the rice growing areas of the world. It has been observed that the lesion development in leaves depends on the compatibility between the host pathogen system (Buddenhagan and Reddy 1972). Moreover chloroplast is mostly affected in a compatible host pathogen interaction resulting into early symptom expression and yield loss (Mohiuddin and Kauffman, 1975). In the present investigation the nature of chloroplast of rice cultivars varying in their degree of susceptibility to BB with virulent cells of *X. O.* pv *oryzae*. Also, the effect of a reducing agent was evaluated in suppression of electrolyte leakage from the chloroplasts infused with the bacterial.

MATERIALS AND METHODS

Chloroplasts of the two rice cultivars TNI (highly susceptible to BB) and IR 20 (least susceptible to BB) were isolated by using the method suggested by Vive Kenandan and Guanam (1975). Cells of *X.O.* pv. *oryzae* scrapped from 48th old culture in Potato sucrose agar media were suspended in sterile distilled water and adjusted to 10^8 colony forming units (CFU)/ml. One ml of this suspension was added to nine ml of the isolated chloroplasts in sterile test tube and mixed thoroughly to study the effect of bacterial infusion on electrolyte leakage from rice chloroplasts.

In another set of experiment the effect of a reducing agent (sodium thio-sulphate) on the intensity of electrolyte leakage from bacteria infused chloroplasts of both the rice cultivars was evaluated. For this aquous solution of sodium thio-sulphate was prepared in two different concentration (*i.e.* 5 and 10 percent). From each of the concentration 1 ml were added to 9.0 ml of the bacteria infused chloroplast suspension respectively maintained in separate test tube. For thorough mixing the tubes were shaked for 10 minutes in a mechanical stirrer. A control was run simultaneously where in no reducing agent and bacteria were added. The tubes were incubated at 28±1°C for 0, 2, 6, 12, 24, 48, 72 and 96h. The conductance of ambient solution in each test tube was measured following the methods of Chopra and Kanwar (1976). The conductivity value in micromhos was obtained by multiplying the conductance and cell constant (K) value (Magyaresy and Buchanon, 1975).

RESULTS AND DISCUSSION

The extracted chloroplast of both susceptible (TNI) and resistant (IR20) rice cultivars when infused with virulent cells of *X.O.* pv oryzae exhibited leakage of electrolytes signifiying damage of photosynthetic apparatus (plate 1 and 2). The electrolyte leakage was highest with chloroplast of susceptible rice cultivars (Table 1). The extracted chloroplast of both the cultivars incubated for different time intervals showed a linear increase in conductivity value. Similarly, chloroplast bacteria combination of the cultivar TNI showed increased in conductivity value upto 72h after incubation. On the otherhand chloroplast of cultivar IR20 when infused with cells of *X.O. oryzae*, the electrolyte leakage was significantly lower as compared to leakage in TNI. The conductivity value in the susceptible and resistant cultivars after 72h incubation was 99.77 and 45.35 micromhos respectively. However, the conductivity value in both the cultivars declined after 96h

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Plate 1 a-b Isolated chloroplast of rice cultivar TNI inoculated with bacterial suspension (*X.O.* Pv. *oryzae*) after 72h of incubation.
a- Chloroplast membrane, disrupted, b- Chloroplast membane disrupted releasing inner content plastids.

of incubation. The introduction of a reducing agent (sodium thio-sulphat) at the rate of 5 and 10 percent respectively to the bacteria infused chloroplast of both the cultivars supressed electrolyte leakage.

The action of reducing agent was more significant in case of cultivar IR20. Moreover with increase in the concentration of reducing agent there was an enhanced restriction in the electrolyte leakage from the bacteria infused chloroplasts of the cultivars. (Table 1).

The trend of electrolyte leakage observed in both susceptible and resistant rice cultivars, it can be concluded that bacteria act in cellular level damaging chloroplast membrane and allowing the electrolyte to leakout. This observation are in confirmatory with earlier findings. Goodman (1972) also reported that membrane damage and electrolyte leakage were consequence of hypersensitive reaction (HR). The level of electrolyte leakage from chloroplasts was found to vary with the degree of susceptibility of the crop cultivar and period of incubation. This finding is in agreement with studies of Cook and Stall (1968). They reported that electrolyte leakage depends on several factors like concentrations of inoculum, age of the host, incubation time and genetic make up of crop cultivar. Introduction of reducing agent to bacteria infused chloroplasts resulted reduction of electrolyte leakage. This may be due to inhibitory effect of the reducing agent against bacterial activity limiting the degree of chloroplast disruptiion in the host (Goodman, 1972).

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Table 1: Electrical conductivity value (micromhos) of the treatment at different time intrvals for both susceptible and resistant cultivars.

Cultivars		Treatments	Electrolyte in micromhos								
			Time in hours								
			0	2	6	12	24	48	72	96	Mean
TNI	1	Chloroplast + Bacteria	0.97	11.25	30.72	53.82	71.76	90.70	99.77	91.09	56.26a
	2.	Chloroplast + Bacteria Reducing agent 5 percent	0.62	6.55	6.98	10.58	11.78	15.06	12.56	8.65	9.09b
	3.	Chloroplasts + Bacteria	0.59	5.81	5.95	7.46	9.78	12.55	10.84	6.55	7.44b
	4.	Chloroplast alone	0.46	1.01	1.12	1.12	1.46	1.58	1.59	1.49	1.23c
		Mean	0.66^{f}	6.15^{e}	11.19^{d}	18.24^{cb}	23.69^{b}	29.97^{a}	31.19^{a}	26.94^{b}	18.50
IR20	1.	Chloroplast + Bacteria	0.86	3.51	10.62	17.74	26.91	36.19	45.35	36.04	22.15^{a}
	2.	Chloroplast + Bacteria Reducing agent 5 percent	0.58	4.82	4.91	5.16	3.86	3.98	3.86	2.98	3.76^{b}
	3.	Chloroplast + Bacteria + Reducing agent 10 percent	0.57	3.86	3.86	4.89	3.96	4.12	3.72	2.55	3.44b
	4.	Chloroplast alone	0.56	0.95	1.17	1.12	1.35	1.46	1.51	1.36	1.18^{c}
		Mean	0.64^{f}	3.23^{e}	5.14^{d}	7.22^{c}	9.02^{cb}	11.43^{b}	13.61^{a}	10.73^{b}	7.63

CD (0.05%) for TNI : Treatment 15.11, hours 4.02
for IR20 : Treatment 11.69, horus 1.89
for two variety 6.01

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