Indian J. mycol. Res., 16 (2) 187-193 (1978)

EFFECT OF X-IRRADIATION ON LEAF PHNOLICS AND RESISTANCE OF JUTE CULTIVARS TO ANTHRACNOSE DISEASE

By

R. P. PURKAYASTHA AND CHANDI RAY

Department of Botany, University o Calcutta, 35, Ballygunge Circular Road Calcutta-7000 19

Effects of X-irradiation on leaf phenolics and resistance of jute cultivars to anthracnose disease were studied. Air dried seeds were treated with different doses of X-rays (10,000 to 70,000r). Those treated with 70,000r showed maxium reduction in both root and shoot lengths. Of the two cvs. JRC 1108 was significantly more affected than JRC 412. JRC 412 was susceptibie to *Colletotrichum corchorum* before and after X irradiation (40,000r) while JRC 1108 became more resistant after treatment, Total phenol content increased in the treated cultivars but markedly in JRC 1108, Increase in phenols in both treated and untreated, infected leaves were also noted. Four and seven phenolic compounds were detected by TLC in leaf extracts of untreated JRC 412 and JRC 1108, respectively. Low concentrarion (0.1 μg/ml) of 4 identified leaf phenolics stimulated mycelial growth of *C, corchorum* while higher concentrations (100 and 500 μg/ml) inhibited growth. Maximum inhibition was observed for guaicol.

INTRODUCTION

X-irradiation sometimes significantly affects the cellular metabolism of plants (Sissakian, 1956; Gunckel and Sparrow 1961; Sen, 1964) including synthesis of phenols. The reports implicating phenolics with disease resistanee of plants are also gradually accumulating. In 1963 Kuc suggested that the

great variety of phenolic compounds occurring in plants might provide a partial explantation for the specificity of host parasite interaction (Tarr, 1972). Since significance of phenolic substances in host parasite interaction has many facets it requires a critical study. The proposed investigation is intended to examine whether X-irradiation has any effect on phenoi metabolism as well as on the resistance of jute cultivars to anthracnose disease caused by *C. corchorum*.

MATERIAL AND METHO

Dried jute seeds of JRC 412 and JRC 1108 were treated with X-rays (40,000r). The treated and untreated seeds were surface sterilized with 0.1% $HgCl_2$ and after thorough washing with sterile distilled water they were sown in non-infested soil. Six weeks old plants were inoculated with spore suspension ($2x10^6$ spores/ml) and incubated at 28-34°C in daylight. Disease intensity was measured as described by Purkayastha and Ray (1977).

For extraction of phenols the method of Sircar and Sircar (1971) was followed with modifications. Known quantity of leaves were plunged into boiling alcohol and the extract was decanted. The leaves were homogenised in fresh ethanol in a blender and the slurry was boiled. Both slurry and decanted liquid were combinded, heated and filtered. The chlorophyll was removed from the alcoholic extract and the alcoholic fraction was evaporated in a rotary film evaporator. Known volume of water was added to the concentrated extract and acidified with N. HCl to pH 4.5. Finally, it was extracted thrice with equal volume of solvent ether and the combined extract was evaporated to complete dryness. The residue was dissolved in known volume of ethanol and diluted with distilled water Total phenol was estimated according to the method of Folin-Denis (A.O.A.C., 1955). Ten ml of the diluted phenol extract was taken and 0.5ml Folin-Denis reagent was added with 1ml of 35% saturated Na_2CO_3 solution. After half an hour blue colour developed

and the changes in colour intensities were recorded by Klett Summerson Colorimeter using a red filter. Results are expressed as mg/g fresh weight of leaves after comparing with authentic phenol (gallic acid equivalent).

EXPERIMENTAL

Effect of X-rays on seedling growth

Different doses of X-rays (10,000, 20,000, 30 000, 40,000, 70,000 r) were applied to dry seeds of JRC 412 and JRC 1108 and after surface sterilization allowed to germinate in Petri dishes at 30 ± 1°C in diffused light. The seedling growth was measured after 72hr of incubation. Results (Table 1) reveal that root and shoot lengths were significantly inhibited at 40,000r and 70,000 r. JRC 1108 is much more sensitive to X-rays than JRC 412.

Table 1 Effect of different doses of X-irradiation on root and shoot lengths of different cultivars of jute *(Corchorus capsularis* L.)

X-ray doses	cv. JRC 412		cv. JRC 1103	
	*Root length (mm)	Shoot length (mm)	Root length (mm)	Shoot length (mm)
Untreated (Control)	46.0	47.7	28.4	31.8
10,000r	47.9	38.1	30.5	32.4
20,000r	39.1	38.7	24.0	25.1
30,000r	37.0	36.9	21.7	23.0
40,000r	36.8	33.8	7.6	18.3
70,000r	28.9	23.9	6.2	12 0

*Average lengths 20 roots and 20 shoots/treatment measured after 72 hr. of incubation. Temp. 30± 1°C.

Effect of X-rays on the development of anthracnose disease

Untreated and X-ray treated (40,000r) seeds were sown in earthenware pots. After 6-weeks, the plants were inoculated, incubated and the disease index was

measured as described. Results are given in Table 2. It appears from the table that the susceptibility of JRC 412 remained unaltered even after X-ray treatment but JRC 1108 which was comparatively less susceptable to anthracnose before treatment became significantly more resistant after X-ray treatment (abuot 30%).

Table 2 Effect of X-irradiation on disease development (*C. corchorum* on jute cvs.)

Cultivars	** Disease index/plant	
	Untreated	X-irradiated *
JRC 412	90.45	85.87
JRC 1103	52.16	36.83

* Seeds irradiated with X-rays (40,000r)

** 96 hr. after inoculation.

Comparison of phenolics of X-irradiated non-irradiated jute leaves (JRC 412 and JRC 1108)

Total phenols were estimated following the method of Folin-Denis (A.O.A.C., 1955). The results are given in Table 3 It was observed that non-irradiated JRC 1108 contained comparatively more phenols than cv. JRC 412. Increase in the quantity of phenols was also greate in X-irradiated JRC 1108

Table 3 Comparison of phenolics of non-irradiated and X-irradiated jute cultivars (before and after infection)

Cultivars	*Average phenol content (mg/g fresh wt. of leaves)			
	Non-irradiated		X-irradiated	
	Healthy	**Infected	Healthy	Infected
JRC 412	0.88	0.92	1.04	1.15
JRC 1108.	1 11	1.23	1.75	2 44

* Baased on 3 replicates

** 4-day old infection

than X-irradiated JRC 412 in relation to their controls. Besides, total phenols, in tividual phenolic compounds of leaves of the said two cultivars were also compared. Ten grammes (fresh wt.) of leaves were collected at random from 6-week old plants and phenolics were extracted with organic solvent following the method described earlier. Drops (10 μl) of phenolac extracts (in 90% ethanol) were added to TLC plates containing silica gel G and developed in benzene-methanol-glacial acetic acid (45 : 8 : 4) and different spray reagents (diazotised p-nitroaniline, bromophenol, Folin-Denis reagent) were used for detection of individual phenolic substance. The details are given in Table 4. Results revealed that JRC 1108 and JRC 412 contained respectively 7 and 4 phenolic compounds. Ferrulic acid, gallic acid and guaicol were detected in both the cultivars. Gentisic acid appeard in JRC 1108 only. Besides, 3 unidentified phenolice (Rf. 0 75, 0.49 and 0.61.) in JRC 1108 and one (Rf. 0 61) in JRC 412 were also detected.

Table 4 Estimation of phenolid sunstances in jute leaves (JRC 412 and JRC 1108)

Phenolic compounds	Amount		Colour in day light	Colour in UV light	Folin-Denis reagent	Diazotized p-nitro aniline	Bromo-phenol
	JRC 412	JRC 1108					
Guaicol (Rf. 0.72)	+	+	nil	violet grey	blue	pink	-ve
Ferrulic acid (Rf. 0.58)	++	+++	light green	blue	—do—	bluish yellow	yellow in blue background
Gallic acid (Rf. 0.23)	++	+++	—do—	reddish brown	—do—	pink	—do—
Gentisic acid (Rf. 0.41)	—	++	nil	light blue	—do—	—do—	—do—
Unknown ompound (Rf. 0.61)	++	++	nil	—do—	—do—	purple	—do—
Unknown compound (Rf. 0.49)	—	++	nil	violet	—do—	—do—	—ve
Unknown compound (Rf. 0.75)	—	+	nil	white	—do—	—do—	—ve

— = nil, + = very small, ++ = small, +++ = fair.

Effect of different concentrations of phenolics (datected in jute leaves) on the mycelial growth and sporulation of C. corchorum

Different concentrations (0.1, 1.0, 1, 10 100 and 500 pp^m) of the phenolics (gallic acid, ferrulic acid, gentisic acid and guaicol) were tested on the mycelial growth of *C. corchorum vitro*. Basal medium (containing 1% glucose, 0.2% asparagine, 0.1% KH_2PO_4; 0.05% $MgSO_4$; $7H_2O$ and 2 pp^m of Zn^{++}, Mn^{++} and Fe^{+++}) was used as control. The flasks were inoculated and

Table 5 Effect of different concentrations of phenolic substances (detected in jute leaves) on the growth of *C. corchorum*

Conc. (ppm)	*Dry wt. of mycelia (mg)			
	Ferrulic acid	Gallic acid	Guaicol	Gentisic acid
0.1	132.00	160.00	142.00	147.33
1.0	107.33	156.33	121.66	139.66
10	103.00	151.00	112.33	101.33
100	92.00	141.00	82.00	96.00
500	85.66	120 00	31.00	62.00
Control (Basal medium without any phenolic compound)	125.33	—	—	—

*Average of 3 replicates/treatment.

incubated at 25 ± 1°C for 10 days. The mycelia were dried for 96 hrs at 60°C. Rusults (Table 5) show that tested phenolics stimulated the growth of the pathogen at lower concentrations (up to 1 ppm) but inhibited growth at higher concentrations (10-500 ppm). Sporulation was, however, totally inhibited in all cases excepting in gallic acid.

Discussion

It is evidient from the result that X-irradiation altered the phenol metabolism of both fha jute cultivars but markedly in case of JRC 1108. About 57% increase

of phenolics was noted in X-irradiated JRC 1108. It is significant to note that unlike JRC 412, the resistance of JRC 1108 (to anthracnose) also increased to a considerable extent after X-ray treatment. It is not yet clearly understood as to whether the greater resistance of X-irradiated JRC 1108 is associated with the increased phenolics (pre-existent) of host or any new host-parasite interaction product. The presence of three unidentified phenolics in JRC 1108 might also be associated with the disease reaction. Although apparently it appears that more phenolics are associated with the greater resistance of JRC 1108, this explanation cannot be totally accepted at this stage since all the identified leaf phenolics stimulated growth of *C. corchorum* at a lower concentration (0.1ppm) and inhibited growth at higher concentrations (100 and 500ppm). Besides, the capacity of a compound to inhibit growth of the fungus *in vitro* does not always relate to its capacity to inhibit the growth of the organism in the host tissues. Guaicol which was found to be most inhibitory to the growth of *C. corchorum* was present in both susceptible and resistant cultivars.

REFERENCES

A.O.A.C. 1955. Official method of analysis of the association of official agricultural chemists. 8th Edn. 144pp. Washington 4 D. C 1965.

Gunckel, J.E. and Sparrow, A.H. 1961. Ionizing radiations : Biochemical, physiological and morphological aspects of their effects on plants. *Encyclopedia of plant physiology* **16** : 555-612.

Purkayastha, R.P. and Ray. Chandi 1977. Effects of foliar application of plant hormones and mineral nutrition of host on the development of anthracnose disease of jute. *Zeitschrift für pflanzenkrankheiten and Pflanzenchutz*. **84** (4) : 193-201.

Sen, S. 1964. Some leaf mutants of jute and their free amino acid pottern. *Bull. Bot. Soc. Beng.* **18** : 77-82.

Sissakian, N.M. 1956. On the nature of changes in metabolism under irradiation effects. Proc. Intn. Conf. on peaceful uses of atomic energy. New York, *United Nations Bull.* **8**. 248-255.

Tarr. S.A.J. 1977. *The principles of plant pa ology*. The MacMillan press, London, pp. 632.