Phenolic Content of Sugarcane in Relation to Red Rot Disease

Following WALKER's[1] demonstration of phenolic compounds as factors for resistance in coloured onions to smudge and neck rot diseases, many more workers have demonstrated the importance of such compounds in the resistance of plants to parasitic diseases. Observations by ABBOTT[2] and PARTHASARATHI and VIJAYASARADHY[3] indicated that the juice of sugarcane varieties resistant to red rot disease had higher phenolic content than that of the susceptible ones. In an attempt to study the nature of resistance in sugarcane to nodal infection by the red rot pathogen, *Colletotrichum falcatum* Went (*Glomerella tucumanensis* (Speg.) ARX and MULLER), the phenolic contents in the node, internode and leaf-sheath of 4 susceptible (Co. 312, Co. 331, Co. 445 and C. 1181) and 2 moderately resistant varieties (Co. 285 and Co. 1070) were determined and the results are reported here.

The tissues of the 8th node from top, between the point of attachment of the leaf-sheath and the growth ring; the leaf-sheath attached to the 8th node (10 cm length from the base) and the internode between the 8th and 9th node (4 cm portion from the middle) of 5 canes of each variety were used for phenol extraction. Extracts were prepared by the method of ECHANDI and FERNANDEZ[4]. The phenol content of the extracts was estimated colorimetrically in chlorogenic acid equivalents, by adopting the Hoepfner-Vorsatz test described by REEVE[5]. To 10 ml of the extract were added 2 ml of 10% freshly prepared sodium nitrite, 2 ml of 10% acetic acid and after 3 min 4 ml of 2*N* sodium hydroxide. The optical density of the solution was then measured in a Bausch and Lomb colorimeter, using a 420 nm filter. The concentration of phenols in the solution was calculated from a standard curve prepared by using the optical densities of different concentrations of pure chlorogenic acid in distilled water. The data are presented in the Table.

The results indicate that the phenolic contents in the node, internode and leaf-sheath of sugarcane varieties moderately resistant to red rot infection are higher than those of the susceptible ones. The nodal tissues of moderately resistant varieties contained $1^1/_2$ times more phenols than the susceptible ones. Since the red rot fungus infects sugarcane chiefly through the tissues at the nodal region of the plant (BUTLER and HAFIZ KHAN[6], CHONA[7], and STEIB and CHILTON[8]), it is felt that the quantity of phenols in the nodal tissues can be used as an index for assessing the degree of resistance to infection by the pathogen and to eliminate the highly susceptible varieties. The phenolic content test is easy to conduct and can be employed in place of the cumbersome and time-consuming nodal inoculation test which is being done currently for the selection of red rot resistant sugarcane varieties.

Zusammenfassung. Hohe Konzentrationen an phenolischen Substanzen in Zuckerrohrpflanzen hemmen die durch den Parasiten *Collectotrichum falcatum* Went verursachte Pilzfäule.

Phenol content in the node, internode and leaf-sheath of sugarcane varieties

Variety	Phenol content of (mg/100 g fresh weight)			Average
	Node	Internode	Leaf-sheath	
Co. 445	30.00	15.50	22.00	22.50
Co. 331	41.00	15.83	22.50	26.68
Co. 1181	42.50	16.50	22.50	27.06
Co. 312	39.33	15.83	25.50	26.89
Co. 1070	64.66	21.00	33.50	39.94
Co. 285	66.33	23.00	34.50	41.33
Average	47.33	18.01	26.75	

S.Em for variety – 0.22 F-test significant
C.D. (at 5% level) – 0.62

22.50 26.68 26.89 27.06 39.94 41.33

S.Em for Part – 0.15 F-test significant
C.D. (at 5% level) – 0.42

18.01 26.75 47.33

K. I. WILSON[9] and D. N. SRIVASTAVA[10]

Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi (India), 10 June 1970.

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[9] Plant Pathology Division, Agricultural College and Research Institute, Vellayani (Kerala, India).
[10] Deputy Agricultural Commissioner, I.C.A.R., Krishi Bhavan, New Delhi (India).

Effects of Ions and Cortisol on RNA Synthesis in Lysed Rat Liver Nuclei

In previous papers we have shown that cortisol enhances RNA polymerase activity of isolated rat liver nuclei by increasing the template activity of the chromatin[1,2]. In these and other reports, attention has been called to the importance of ionic conditions for RNA synthesis in in vitro systems[3–6]. In order to elucidate the mechanism by which cortisol controls the RNA synthesizing machinery of the cell nucleus, it seems necessary to investigate the ionic dependence of the hormonal effect and the nature of the product synthesized.

Male Wistar BR II rats (120–160 g) were used. The ^{14}C-labelled nucleoside triphosphates were obtained from The Radiochemical Centre, Amersham; the non-labelled ribonucleoside triphosphates, creatine phosphate and

creatine phosphokinase, as well as the ribonucleoside monophosphates from Boehringer, Mannheim. RNA polymerase from *E. coli* was prepared by the method of ZILLIG et al.[7]. DNA from rat liver was prepared by the method of MARMUR[8] with an additional pronase-ribonuclease treatment before the last precipitation with isopropanol.

Rat liver nuclei were prepared in isotonic sucrose and purified by treatment with Triton X-100 or Nonidet NP 40 as previously described[9]. In the experiments in which the hormonal effect was studied, the intact nuclei were incubated for 10 min at 37 °C in the presence of cortisol dissolved in ethanol or solvent alone. The final concentration of ethanol did not exceed 2%. The cortisol concentration was 5 µg/ml[2].

For the measurement of RNA polymerase activity, the nuclear sediment, obtained after hypotonic lysis of the purified nuclei in 0.05 *M tris*-HCl buffer, pH 7.4, for 10 min, followed by centrifugation at 8000 × *g* for 10 min, was used. The pellet resuspended in 0.065 *M tris*-HCl buffer, pH 7.9, by hand homogenization was used for further characterization. DNA was determined by the method of CERIOTTI[10], protein according to LOWRY et al.[11] and RNA according to OGUR and ROSEN[12]. The in vitro system for the assay of RNA polymerase activity was essentially the same as described by LUKACS and SEKERIS[1]. Amounts of nuclear sediment containing 100 µg DNA were used. Determination of the template activity of the nuclear sediment was performed in the presence of an excess of RNA polymerase as described by BEATO et al.[2]. The concentration of ions will be indicated in the text of the figures.

For base analysis of the RNA, incubation mixtures were prepared containing all 4 ribonucleoside triphosphates labelled with ^{14}C together with creatine phosphate, creatine phosphokinase and the different ions. The RNA was precipitated with cold 5% $HClO_4$, washed and hydrolyzed in 0.5 *N* KOH. The hydrolysate was chromatographed by the method of LANE[13] and the mononucleotide spots counted by liquid scintillation.

Optimal rate of RNA synthesis was observed at 37 °C and pH 7.9. The incorporation of ^{14}C-uridinetriphosphate (^{14}C-UTP) into acidinsoluble material requires the presence of the other 3 ribonucleoside triphosphates and divalent cations, and can be markedly inhibited by actinomycin D or ribonuclease treatment.

The incorporation of ^{14}C-UTP into RNA by the nuclear sediment was optimally stimulated by $MnCl_2$ at a concentration of 3.6 m*M* (Figure 1). With $MgCl_2$ as divalent cation maximal incorporation was observed at 15–20 m*M* and reached only 60% of that seen with optimal $MnCl_2$ concentration. Optimal expression of the stimulatory effect of cortisol on RNA synthesis was seen in the presence of 2.5–10 m*M* $MnCl_2$ and was about 30%. In the presence of $MgCl_2$ the hormonal effect was only seen in a concentration range between 7 and 12 m*M* and the variability of experimental results was greater than in the presence of $MnCl_2$ (Figure 1). When both divalent cations were used together, the expression of hormonal stimulation tended to disappear. A very similar dependence on divalent cation concentration was seen when the template activity of the nuclear sediments was tested in the presence of an excess of bacterial RNA polymerase from *E. coli*.

Addition of increasing amounts of ammonium sulfate to the RNA polymerase assay results in a very pronounced stimulation of the RNA synthesis, especially in

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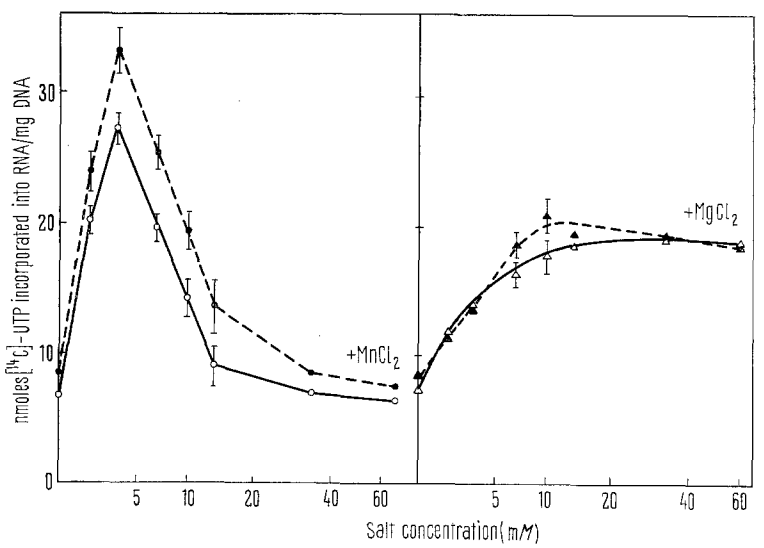

Fig. 1. Effect of divalent cations on the RNA polymerase activity of rat liver nuclear sediment. RNA Polymerase was determined[2] at different concentrations of $MnCl_2$ or $MgCl_2$, in the nuclear sediment from control (—) and cortisol-treated nuclei (---). Incubation was performed at 37 °C for 10 min. The points are the mean of 5 duplicate experiments. For this and the following figure, standard deviations are only shown when the difference between control and hormone treated preparations is significant.

the presence of Mn^{2+} (Figure 2). This could be the consequence of an inhibition of ribonuclease activity[14] or of a dissociation of the DNA-protein complex due to the high ionic strength[15]. A direct effect of ammonium sulfate on the RNA polymerase seems unlikely, since maximal stimulation was seen at 0.2–0.4 *M*, a concentration which inhibits the activity of the purified liver enzyme when tested with isolated DNA as template[16]. As has been suggested[2,16], the stimulation of RNA synthesis induced by cortisol in the presence of an excess of bacterial RNA polymerase is probably due to the liberation of new regions of the template. Since high salt concentrations also demask the DNA in the chromatin[15], the hormonal effect was not observed when the test was performed at high concentrations of ammonium sulfate (Figure 2).

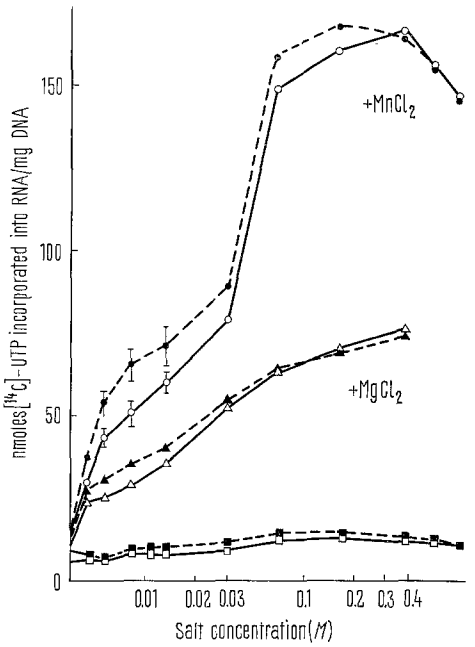

Fig. 2. Effect of ammonium sulfate on the RNA polymerase activity of rat liver nuclear sediment. RNA polymerase activity was determined at variable concentrations of ammonium sulfate (abscissa) alone (□ ■), and in the presence of 6.6 m*M* $MnCl_2$ (○ ●) or 6.6 m*M* $MgCl_2$ (△ ▲), in the nuclear sediment from control (—) and cortisol-treated (– – –) nuclei. Incubation was performed at 37 °C for 10 min. Each point is the mean of 3 duplicate experiments.

Base analysis of in vitro synthesized RNA

Conditions of incubation	Base analysis (%) A	U	G	C	AU/GC
+ Mn^{2+}					
Control	15.75	26.30	26.65	31.30	0.725 ± 0.023
Cortisol	16.80	29.20	25.40	28.60	0.823 ± 0.029
Actinomycin D (2 µg/ml)	17.45	31.54	26.04	24.97	0.960 ± 0.046
+ Mg^{2+}					
Control	13.35	25.95	30.90	29.80	0.647 ± 0.019
Cortisol	12.10	30.05	24.05	33.80	0.730 ± 0.045

The incubation mixture and base analysis was as described in the text. The concentration of ^{14}C-labelled nucleoside triphosphates was 1.2×10^{-3} *M* (specific activity 1.66 µc/µmole). $MnCl_2$ or $MgCl_2$ were used at concentrations of 6.6 m*M*. The values represent the mean and standard error of 3 separate experiments.

Most of the RNA synthesized in vitro by the RNA polymerase of the nuclear sediment has a sedimentation constant between 4 and 10 S as shown by sucrose density gradient centrifugation. This low molecular weight may result from the action of nucleases or from the absence or inactivity in our system of some factors required in vivo for joining the small RNA fragments to form larger molecules[17]. The sedimentation profile of the RNA was not affected by the ionic conditions of the synthesis nor by the preincubation of the nuclei with cortisol. Moreover, a similar sedimentation pattern was observed when the synthesis was performed in the presence of an excess of bacterial RNA polymerase.

The RNA synthesized by the rat liver nuclear sediment in vitro shows a rather low adenine + uracil/guanine + cytosine (AU/GC) ratio, between 0.7–0.8 depending on the ionic conditions (Table). RNA synthesized in the presence of Mg^{2+} has a lower AU/GC ratio than that synthesized in the presence of Mn^{2+}, while this ratio rises in the presence of high concentration of ammonium sulfate. Incubation of the nuclei for 10 min with cortisol both in the presence of Mn^{2+} or Mg^{2+} results in a 10–15% increase of the AU/GC ratio of the RNA synthesized by the nuclear sediment (Table). This is in accord with our previous finding of the messenger properties of the nuclear RNA induced by cortisol in vivo and in vitro[18] as well as with reports by YU and FEIGELSON[19].

These experiments show that the concentration of manganese, magnesium and ammonium sulfate are of the utmost importance for the manifestation of the hormonal effect on nuclear RNA synthesis and that an early response of rat liver nuclei to cortisol is the stimulation of the synthesis of DNA-like RNA[20].

Zusammenfassung. Die Inkubation von isolierten Rattenleberzellkernen mit Cortisol führt zu einer Steigerung der endogenen Polymeraseaktivität um 30% im Kernsediment, wenn bestimmte Ionenbedingungen eingehalten werden. Cortisol bewirkt einen Anstieg im AU/GC Verhältnis der in vitro synthetisierten RNA ohne das Sedimentationsprofil in Saccharosedichtegradienten zu beeinflussen.

M. BEATO[21], J. HOMOKI,
D. DOENECKE[21] and C. E. SEKERIS

Physiologisch-Chemisches Institut,
Philipps-Universität, D-335 Marburg (Germany),
6 April 1970.

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[21] Fellow of the Deutsche Forschungsgemeinschaft.