EXPERIENTIA

Vol. 27 – Fasc. 2 Pag. 121–240 15. 2. 1971

SPECIALIA

Shock Effects on Plants: Tannic Acid and Chlorogenic Acid in Yam Roots

The accumulation of polyphenols is reported to be a non-specific response to injury in plants[1,2]. Both tannic acid and chlorogenic acid are secondary metabolites with phenolic nuclei and are widely distributed in the plant kingdom[3,4]. Although the greatest concentration of tannins appears to occur in dead or dying cells, their protein-binding capacity may be important in these cells, or cells which have a high concentration of tannins, as a defense mechanism in aiding the resistance of such cells to attack by plant pathogens[5]. Accumulation of chlorogenic acid may also be important as a defense mechanism by hastening lignification processes[6]. Both tannic acid and chlorogenic acid were measured in shocked yam roots in order to determine the magnitude and duration of the injury response after shock treatment, where shock is defined as a fast-rising pressure pulse of 4 sec duration.

Materials and methods. Yam tuber halves were shocked 3 days after wetting in an air-loader, previously described[7], from 10 to 44 pounds per square inch (ψ) in 10 ψ increments (0.66–2.66 kg/cm^2, respectively). The tuber halves were set on large vials of water, under continuous low-level illumination from a cool white fluorescent tube. Whole roots were harvested at weekly intervals after shock treatment for 4 weeks. The extraction procedure for chlorogenic acid of ZUCKER and AHREN[8] was followed. Tannic acid was determined by the Folin-Denis method of ROSENBLATT and PELUSO[9], and was read at 650 nm in a Coleman-Hitachi spectrophotometer.

Results and discussion. Both phenolic substances accumulated after shock treatment (Tables II and IV). However, there was essentially no significant change in their concentrations over the 4-week-period, indicating a somewhat immediate accumulation in response to shock treatment.

Extensive histological studies on wheat, rice, radish and pea roots indicate that the degree of tissue damage, including lesions, is somewhat proportional to shock level. On the basis of these studies, one would expect to find increasing amounts of tannic acid with increasing shock levels, but an inverse relationship was actually

Table I. Data showing shock effects and the effect of post-shock weekly intervals on the tannic acid content (mg/100 mg) of yam roots

Time (weeks)	Control	Shock level (ψ)				Totals
		10	20	30	44	
1	0.191	0.230	0.130	0.230	0.122	0.903
2	0.130	0.314	0.091	0.264	0.162	0.961
3	0.142	0.304	0.050	0.253	0.304	1.053
4	0.092	0.396	0.079	0.274	0.345	0.186
Totals	0.555	1.244	0.350	1.021	0.933	4.103

Table II. Analysis of variance and least significant differences for the tannic acid data

Variation source	df	SS	MS	VR	95% LSD
Time effect	3	0.009	0.003	0.75	
Shock effect	4	0.131	0.033	8.25[a]	0.194
Error	12	0.49	0.004		

[a] F = 99.5%.

Table III. Data showing shock effects and the effect of post-shock weekly intervals on the chlorogenic acid content (μmoles/100 mg) of yam roots

Time (weeks)	Control	Shock level (ψ)				Totals
		10	20	30	44	
1	0.42	0.62	0.28	0.76	0.53	2.61
2	0.24	0.85	0.15	0.62	0.56	2.42
3	0.55	0.82	0.09	0.55	1.08	3.09
4	0.32	0.12	0.18	0.97	0.97	2.56
Totals	1.53	2.41	0.70	2.90	3.14	10.68

[1] C. C. CRAFT and W. V. AUDIA, Bot. Gaz. *123*, 211 (1962).
[2] G. JOHNSON and L. A. SCHAAL, Am. Potato J. *34*, 200 (1957).
[3] S. A. BROWN, in *Biochemistry of Phenolic Compounds* (Ed. J. B. HARBOURNE; Academic Press, New York 1964), p. 361.
[4] A. C. NEISH, in *Plant Biochemistry* (Eds. J. BONNER and J. E. VARNER; Academic Press, New York 1965), p. 581.
[5] T. SWAIN, in *Plant Biochemistry* (Eds. J. BONNER and J. E. VARNER; Academic Press, New York 1965), p. 552.
[6] T. T. MCCLURE, Am. J. Bot. *47*, 277 (1960).
[7] S. A. MURRAY, Experientia *26*, 319 (1970).
[8] M. ZUCKER and J. F. AHREN, Pl. Physiol. *33*, 246 (1958).
[9] M. ROSENBLATT and J. V. PELUSO, Ass. Off. Agric. Chem. *24*, 170 (1941).

Table IV. Analysis of variance and least significant differences for the chlorogenic acid data

Variation source	df	SS	MS	VR	95% LSD
Time effect	3	0.0509	0.0170	0.2901	
Shock effect	4	1.0241	0.2560	4.3686[a]	0.24
Error	12	0.7031	0.0586		

[a] F = 97.5%.

observed. A possible explanation for this observation is, since tannins are associated with cytoplasmic organelles[5], that tannins are not formed and, therefore, not stored in broken cells. Broken cells, or lesions, are characteristic in material shocked at 30 and 40 ψ, sometimes occurring after 20 ψ, but have not yet been observed after 10 ψ. Unfortunately, the values obtained for the 20 ψ exposure for both tannic acid and chlorogenic acid are believed to be anomalous because of equipment failure. The reason for this belief is based on the chlorogenic acid result, since the same tuber provided material for both assays. That is, the synthesis of chlorogenic acid is known to be linear with increasing oxygen concentrations reaching a maximum at about 20% and levelling with further increases[10]. Since the shock tube is loaded with air in the constant volume loading chamber, the air gets compressed with higher shock levels. Since the load volume is kept constant, oxygen concentration becomes variable depending on the shock level.

There was a progressive increase in chlorogenic acid with increasing shock levels (Table III), thereby agreeing with ZUCKER and LEVY's[10] work on the effect of oxygen concentrations on chlorogenic acid synthesis in potato tubers. Premature lignification has been observed in shocked pea roots but only after a 40 ψ shock exposure. The roots of tubers for the 30 and 44 ψ shock exposures were slightly brownish, possibly indicating premature suberization of the roots. Chlorogenic acid may also act as a plant growth regulator[11]. Metabolically, it is a competitive inhibitor of IAA-oxidase, and is considered to be effective both in the catabolism and anabolism of auxin[12–14]. It is known that increased concentrations of auxin (or auxin-like substances) have an inhibitory effect on plant growth[15]. Observations that plant growth is reduced after shock treatment[7,16] may possibly be explained by increased concentrations of chlorogenic acid[17].

Résumé. Les tanins de l'acide chlorogénique s'accumulent immédiatement après que les racines d'ignames ont été soumises à des chocs de 10 à 44 ψ (0.6–2.66 kg/cm^2), consistant en une pulsation de la pression comparable à une rafale d'air dans un tube. L'accumulation des tanins est inversement proportionelle à la pression. Mais, l'acide chlorogénique s'accumule progressivement avec élèvation du niveau du choc.

SYLVIA A. MURRAY

1522 Willow Street, Alameda (California 94501, USA),
20 July 1970.

[10] M. ZUCKER and C. C. LEVY, Pl. Physiol. *34*, 108 (1959).
[11] R. L. WAIN and H. F. TAYLOR, Nature, Lond. *207*, 167 (1965).
[12] S. RABIN and R. M. KLEIN, Arch. Biochem. Biophys. *70*, 11 (1957).
[13] S. A. GORDON and L. G. PALEG, Pl. Physiol. *36*, 838 (1961).
[14] P. E. PILET, Phytochemistry *2*, 617 (1964).
[15] D. J. MORRÉ and J. BONNER, Physiol. plant. *18*, 635 (1965).
[16] S. A. MURRAY and C. L. NEWCOMBE, Radiat. Bot. *11*, in press.
[17] Acknowledgment. This work was supported in part by funds from Advanced Research Projects Agency, Department of Defense, through the U.S. Geological Survey, under ARPA Order No. 938. Special thanks are expressed to Dr. C. L. NEWCOMBE of San Francisco State College, and Dr. H. T. SHACKLETTE of the Geological Survey for making this study possible.

Potentiation of Haemolysis by the Combined Action of Phospholipase A and a Basic Peptide Containing S-S-Bonds (Viscotoxin B)

Although red cells contain lecithin in their membranes and are susceptible to lysis by lysolecithin, they are not lysed by phospholipase A. However, in the presence of a basic peptide fraction of cobra venom, the direct lytic factor (DLF) which is a weak haemolysin by itself, phospholipase A becomes strongly haemolytic and cleaves membrane phospholipids[1]. Recent results from this institute have indicated that the action of DLF depends on the presence of disulphide bridges[2,3]. It was further suggested that the potentiating effect is due to an alteration of the membrane structure caused by interaction of DLF with SH groups of membrane constituents, and that the combination of basic charge with disulphide bonds is a general structural feature of peptides which enable phospholipase A to cause haemolysis[3].

This hypothesis was put to the test with viscotoxin B, one of a group of related peptides which have been isolated from the European mistletoe, *Viscum album* L. These peptides have S–S-bonds and a net positive charge[4]. In an earlier publication, a crude viscotoxin preparation has been reported to cause haemolysis, besides having effects on the heart and circulation[5]. The preparation of viscotoxin B was kindly supplied by Dr. G. SAMUELSSON, Stockholm. Phospholipase A was separated from bee venom according to the procedure of HABERMANN and REIZ[6]. Heparinized guinea-pig blood was centrifuged and the packed cells were washed 3 times with 1% NaCl solution. They were finally suspended in 0.01 M phosphate buffer pH 7.3 containing 0.15 M NaCl and 0.45 mM $CaCl_2$ (20 times the original blood volume). Each 0.25 ml of phospholipase A solution (2×10^{-5} g/ml) and of various concentrations of viscotoxin B were added to 4.5 ml red cell suspension. The

[1] E. CONDREA, A. DE VRIES and J. MAGER, Biochim. biophys. Acta *84*, 60 (1964).
[2] P. PATZER and W. VOGT, Naunyn-Schmiedebergs Arch. Pharmak. exp. Path. *257*, 320 (1967).
[3] W. VOGT, P. PATZER, L. LEGE, H.-D. OLDIGS and G. WILLE, Naunyn-Schmiedebergs Arch. Pharmak. *265*, 442 (1970).
[4] G. SAMUELSSON, T. OLSON, T. MELLSTRAND and B. PETTERSON, Second International Symposium on Animal and Plant Toxins, Toxicon, *8*, 150 (1970).
[5] H. F. ZIPF, Arch. exp. Path. Pharmak. *209*, 165 (1950).
[6] E. HABERMANN and K. G. REIZ, Biochem. Z. *341*, 451 (1965).