GEOBIOS 21 : 265-268, 1994

SULFHYDRYLS AND LEAF GALL FORMATION IN *CINNAMOMUM TAMALA*

S. C. JOSHI* and P. TANDON

Department of Botany, School of Life Sciences, North-Eastern Hill University, Shillong-793 014, India

(Received September 10, 1993; Revised August 4, 1994)

Key words : Leaf gall, *cinnamomum*, sulfhydryls

ABSTRACT

The gall tissues in *Cinnamomum tamala* contained more sulfhydryls as compared to normal and normal looking portions from diseased leaf. The young gall contained maximum amount which declined with gall maturation. The possible role of sulfhydryl in leaf gall formation is discussed.

INTRODUCTION

The gall formation in plants following infection by differnt pathogens brings about a number of physiological and bio-

* For correspondence : G. B. Pant Institute of Himalayan Environment and Development, Garhwal Unit, HAPPRC, Post Box 14, Srinagar (Garhwal)—246 174.

chemical changes, both qualitative and quantitative at the level of growth hormones, oxidative enzymes, phenolics, sugars, starch, lipids and amino nitrogen (Dixon, 1983; Joshi & Tandon, 1984). Various functions have been attributed to sulfhydryl groups (Harington, 1967, Fahey & Newton, 1986; Wingate et al., 1988). Tumor formations are characterized by excessive cell division and sulfhydryls are invariably associated with cell division (Harington, 1967). Howe er, reports on the contents of sulfhydryls during gall formation in plants are scant. Therefore, the present investigation was undertaken to study the changes in the contents of sulfhydryl groups during gall formation and the possible role of sulfhydryls in leaf gall formation in *Cinnamomum tamala* Fr. Nees.

MATERIALS AND METHODS

(a) *Plant material* : Fresh tissues, from *C. tamala* leaf galls (at different developmental stages, *viz.*, young gall, green mature gall, completely transformed leaf & brown gall) incited by a mite (*Eriophyes* sp.) normal leaf and normal looking portions from diseased leaf, were used as experimental material.

(b) *Extraction and estimation of sulfhydryls* : The tissues were extracted in 3% (W/V) metaphosphoric acid and estimated by the nitroprusside reaction (Grunert & Phillips, 1951). The amount of sulfhydryls was calculated using a standard curve of reduced glutathione and is expressed as mg/g fresh wt of tissue. Method of Tandon & Arya (1982) was used for the measurement of peroxidase activity.

(c) *Oxidation of reduced glutathione in presence and absence of peroxidase and auxin protectors* : To obtain more insight into the possible role of sulfhydryls in gall formation, one experiment using reduced glutathione (this has been reported to occur uniformely in eukaryotes as the major thiol) was conducted. The reduced glutathione (GSH) 0.5 mM was incubated at 20°C in 0.4 M phosphate buffer (pH 5.8) in the presence of 0 1 mM dicholorophenol, 0.50 µg/ml peroxidase (HRP) and 0.1 mM manganese chloride. In another experiment, 0.1 ml of auxin protectors isolated from gall tissues was also added in the incubation mixture. This isolation and characterizations of auxin protectors from leaf gall tissues have already been described elsewhere (Joshi & Tandon, 1989). The destruction of glutathione was followed by withdrawing 1 ml of aliquot of the incubation mixture at various time intervals and assaying by means of nitroprusside reaction.

RESULTS AND DISCUSSION

The gall tissues except brown gall showed higher sulfhydryls contents as compared to normal and normal looking portions from diseased leaf. The young gall contained maximum amount which declined with gall maturation (Table 1). There is an increasing evidence that the juvenile stage/actively dividing stage is associated with the presence of antioxid-

Table 1. Content of sulfhydryls and peroxidase activity in various stages of gall development in *C. tamala*.

Tissue	Amount (mg/g fresh wt)	Peroxidase (△ A/min/g fresh wt)
Normal	2.53 ± 0.04	51.50 ± 3.20
Normal from diseased leaf	2.26 ± 0 08	42.50 ± 4.30
Young gall	3.00 ± 0.06	2.17 ± 0.09
Green mature gall	2.80 ± 0.10	2.18 ± 0.12
Completely transformed leaf	2.72 ± 0.04	2.77 ± 0.06
Brown gall	2.00 ± 0 06	3.10 ± 0.06

± S. E.

ants, in general (Szent-Gyorgyi, 1968) and sulfhydryls gradients in particular (Pilet & Dubois, 1968). Thus the presence of more sulfhydryls in young gall tissue may be resp nsible for its conti uous abnormal growth. On the other hand, the brown gall which represents the last stage of gall development contained less sulfhydryl contents. In any plant cell sulfhydryl levels can be maintained either by increased synthesis or less destruction (Rennenberg, 1982). It has been reported that in the plant system peroxidase enzyme catalyses sulfhydryls contents (Balasimha & Tewari, 1978). Addition of peroxidase enzyme into the incubation mixture of reduced glutathione caused oxidation of reduced glutathione. However, auxin protector substances (O-dihydroxyphenolic in nature) associated with gall tissues inhibited this oxidation (Table 2). Stonier (1972) reported that auxin protectors may be responsible for the accumulation of sulfhydryl contents in tumor tissues. In *C. tamala* leaf gall maximum auxin protection activity was observed in young gall followed by green mature gall, completely transformed leaf and brown gall (Joshi & Tandon, 1989). Further, gall tissues also contained significantly low peroxidase activity as compared to normal and normal from diseased leaf (Table 1). There seems to exist a relationship between auxin protection activity peroxidase activity and contents of sulfhydryl groups in gall formation in *C. tamala*.

Table 2. Percentage inhibition of peroxidase-catalyzed oxidation of reduced glutathione (GSH) in presence and absence of auxin protector-I.

GSH in presence of cofactor	Time (min)					
	5	10	20	30	40	50
No peroxidase	100	100	100	100	100	100
Peroxidase	50	34	18	12	10	10
Peroxidase & auxin protector I	100	100	100	100	100	100

In summary, high concentration of sulfhydryl contents invariably associated with leaf gall tissues of *C. tamala* may be responsible for their abnormal proliferation.

ACKNOWLEDGEMENT

Financial support from CSIR, New Delhi is acknowledged.

REFERENCES

Balasimha, D. and Tewari, M.N. Oxidation of glutathione by peroxidase isoenzymes in fenugreek. *Biol. Plant.*, 1978, 20, 387-391.

Dixon, A.F.G. Insect-induced phytotoxemias : Damage, tumors and galls. In : *Current Topics in Vector Research,* Praeger Publishers, New York, 1983, Vol. 1, pp. 297-314.

Fahey, R.C. and Newton G.L. Occurrence of low molecular weight thiols in biological system. In : *Functions of Glutathione : Biochemical, Physilogical, Toxicological and Clinical Aspects*, (eds.), A. Larssen. Raven Press, New YorK, 1986, pp. 251-261.

Grunert, R.R. and Phillips P.H. A modification of the nitroprusside method of analysis for glutathione. *Arch. Biochem. Biophys.*, 1951, 30 217-225.

Harington, J.S. The sulfhydryl group and carcinogenesis. *Advanc. Cancer Res.*, 1967, 10, 247-309.

Joshi, S.C. and Tandon, P. Studies on certain oxidases, their isozymes and phenols as related to gall formation in *Leea indica*. *Biochem. Physiol Pflanzen* 1984, 179, 711-715.

—. and —. Association of auxin protectors in *Cinnamomum tamala* Fr. Nees leaf gall formation. *Indian J. exp. Biol.*, 1989, 27, 1020-1024.

Pilet, P. E. and Dubois, J. Variations due taux en composes sulphydriels acidosolubles de tissues cultives *in vitro*. *Plant Physiol.*, 1968, 21, 445-454.

Rennenberg, H. Glutathione metabolism and possible biological roles in higher plants. *Phytochem.*, 1982, 21, 2772-2781.

Stonier, T. The role of auxin protectors in autonomous growth. In : *Les Cultures de Tissus de Plantes. Proc. 2nd Intern. Conf. on Plant Tissue Culture*, Strasbourg, France, 1972, pp. 423-435.

Szent-Gyorgyi, A. *Bioelectronics*. Academic Press, New York, 1968.

Tandon, P. and Arya, H. C. Association of auxin protectors, peroxidase, indoleacetic acid oxidase and polyphenol oxidase in *Zizyphus* gall and normal stem tissues grown in culture. *Biochem. Physiol. Pflanzen.*, 1982, 177, 114-124.

Wingate V.P.M., Lawton, M.A. and Lamb, C.J. Glutathione causes a massive and selective induction of plant defense genes. *Plant Physiol.*, 1988, 87, 206-210.