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CUSHION GALLS OF COCOA IN INDIA*

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ABSTRACT

During a survey of cocoa gardens in South India, cushion galls were observed in Trichur District (Kerala State). Based on symptomatology they were identified as fan gall and knob gall. The etiology of these galls are yet to be established. Attempts to transmit these disorders were unsuccessful. In a pod collected from a normal cushion of a fan gall affected tree, only 50 per cent of the beans were normal and healthy. The abnormal beans failed to germinate. Anatomical peculiarities of knob gall and biochemical basis of hyperauxinity in gall tissue were investigated.

INTRODUCTION

Cocoa cushion gall is a serious malady in several cocoa growing countries. Mainly five kinds of cocoa cushion galls *viz.*, green point gall, flowery gall, knob gall, disc gall and fan gall are recognised (Thorold, 1975). Of these, green point gall and flowery gall are more important and occur in epidemic proportions in some of the cocoa growing areas causing serious threat to the cocoa industry (Hutchins, 1959). Though cushion galls of one or the other type have been recorded from other cocoa growing countries, they have not been so far reported from India.

The abnormality has been reported to be due to infection by micro-organisms or high levels of auxins (Stonier, 1972). The present paper examines the symptomatology, etiology, anatomy and some aspects related to auxin metabolism of cocoa plants affected by cushion galls.

MATERIALS AND METHODS

A survey was conducted in the cocoa growing areas during 1979 to study the occurrence of cushion galls, in all the districts of Kerala, six districts of Karnataka *viz.*, Dakshina Kannada, Kodagu, Bangalore, Tumkur, Shimoga and Chickmagalur and Kanyakumari district in Tamil Nadu. One hundred and fifty gardens were covered in this survey.

The field symptoms of cushion galls, morphology of the gall and the percentage of trees affected were recorded.

To study the association if any, of fungi or bacteria, with this disorder, isolations were made on potato dextrose agar (PDA) and nutrient agar from the abnormal cushion or galls.

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Inoculation tests were conducted with the fungi isolated as well as with the tissue macerate, separately and in combination on 6 – 8 year-old healthy cocoa plants. The healthy cushions were inoculated as follows :

(1) Pricking the cushion with fine needles and spraying with mycelial and spore suspension in sterile water, (2) after pricking the cushions as above, gall tissue macerate was applied on the spot, (3) two to three mm deep holes were made with a cork borer on the cushions and similar size gall tissues cut from the abnormal cushions were introduced in each such hole and sealed with cellophane tape, (4) in the holes made as above sporulating mycelial discs cut from the culture of the fungus on PDA was placed and sealed with cellophane tape, and (5) uninoculated but wounded (controls).

The nature of cocoa beans and the percentage germination of the beans in a mature cocoa pod collected from an apparently normal cushion of an affected tree on which more than 50 per cent of the cushions were showing abnormality, were studied. After examining the nature of the beans, the normal and abnormal beans were sown separately in polythene bags. The germination of normal and abnormal beans was recorded two months after sowing.

To study the anatomical features of the galls, materials were fixed in FAA and 12μ m thick sections were taken in rotary microtome following the method of Johansen (1940) and the slides were stained with safranin and fast green.

For enzymatic analysis in the healthy and affected cushions, one gram portions of the tissue was extracted in 10 ml chilled 0.1 M potassium phosphate buffer (pH 6.1). The homogenate was centrifuged at 12000g for 20 min at 0°C and supernatent liquid used for enzyme assays. Peroxidase (EC. 1.11.1.7) was assayed with p-phenylene diamine as hydrogen donor. The reaction mixture contained 4 ml 0.1 M K-phosphate buffer (pH 6.0) 0.3 ml 0.03 M H_2O_2, 0.3 ml 0.1 M phenylene diamine and 1 ml enzyme extract. The reaction was curtailed by the addition of phenylene diamine and read at 30 sec. intervals in Spectronic 21 spectrophotometer at 485 nm. The polyphenol oxidase (EC. 1.14.18.1) assay mixture contained 3 ml K-phosphate buffer (pH 6), 1 ml 0.1 M catechol (in phosphate buffer) and 2 ml extract and read at 495 nm.

For estimation of phenols two grams tissue was extracted in 25 ml hot 90 per cent methanol, cooled and filtered. Total phenols was estimated by Folin-Denis reagent (Farkas and Kiraly, 1962) and the o-dihydric phenols by Arnow's reagent (Mahadevan, 1966).

RESULTS AND DISCUSSION

Out of 150 gardens surveyed, cushion galls were observed only in one garden in Trichur District, Kerala. Two types of galls were observed in this garden. From the appearance of the galls they were designated as fan and knob gall corresponding to the description of Thorold (1975).

Fan gall

The flower cushions of the affected trees produced profusely branched small

stem like outgrowths bearing numerous flowers. From a single cushion 1–10 (usually 5–6) such outgrowths with short internodes were produced. The branches of these outgrowths with the flowers were closely packed and appeared like a loose gall on a cushion (Fig. 1). Affected tree had a few to 110 such abnormal cushions. The length of each such outgrowth was usually 7 – 9 cm, maximum being 25–30 cm. Occasionally small leaves were produced at the tip of the outgrowths resembling that of a fan branch. Such abnormal flowering was observed on 8 per cent of the trees with 3 per cent in initial, 2 per cent in the medium and 3 per cent in the advanced stage of severity. Pod setting was not observed on abnormal cushions. But pod setting was rarely observed on normal cushions of the affected trees. Occurrence of fan galls were reported from New Guinea, Papua and New Britain (Shaw and Burnett, 1969).

Knob gall

Knob galls were observed on 3 per cent of the trees in the affected garden. Affected trees were found to bear 4–12 galls (usually 4–6) on the main trunk below the jorquette. The galls were produced on the cushions as hard, woody swellings with a smooth surface and did not bear flowers. As very few such galls were found on each tree, yield was not markedly affected. Similar type of knob galls were reported from New Britain and New Ireland (Shaw and Burnett, 1969).

Etiology

In the present investigations these two types of galls could not be transmitted to normal flower cushions on gall-free trees. Neither fungi nor bacteria could be isolated from the abnormal galls or cushions. Out of 500 isolations made on PDA, only in two cases *Calonectria rigidiuscula* (Berk and Br.) Sacc. (Conidial state : *Fusarium decemcellulare* Brick) was obtained from fan gall. On inoculation to healthy cushions it did not produce any of the symptoms. Neither the fungus nor the abnormal tissue macerate could induce symptoms on healthy cushions during the period of one year subsequent to inoculations. Hence the etiology of this abnormality remains unknown, and attempts to transmit the disorder was not successful.

In some of the cocoa growing countries, an isolate of *C. rigidiuscula* was frequently isolated from the green-point galls and typical gall symptoms could be produced by inoculating with this fungus as well as by grafting gall tissue on to healthy plants (Thorold, 1975).

Studies on germination of beans

In pods collected from normal cushions of a fan gall-affected tree, only 50 per cent of the beans were normal and healthy, the rest being thin and soft and failed to germinate. But 75 per cent of the normal beans from the same pod germinated. Germination of the beans in the pods from affected trees was only 41 per cent, whereas it was 90 per cent in pods from healthy trees.

Anatomical observation

Fan gall

The T.S./L.S. of the branches of the flowery gall did not show any

anatomical differences from that of the normal stem. Stonier and yang (1971) reported that the phloem fibres failed to differentiate or the differentiated fibres failed to lignify in case of vascular bundles developing in the vicinity of growing crown gall tumors of sunflower. In the present study any such abnormal phloem fibres were not observed.

Knob gall

It was semi-circular in outline both in T.S. and L.S. The vasculature was continuous with that of the trunk on which it was borne (Fig. 2). In the early stages of growth the vasculature showed definite polarity and orientation (Fig. 3) and it branched out as the gall developed and lost its orientation and polarity. Young galls showed a number of protuberances which were groups of highly meristematic parenchymatous cells. It appeared that the gall increases in size by hypertrophy as well as hyperplasia.

The xylary tissue of the mature galls attain two kinds of arrangements-concentric and reticulate. In the former type vessels are concentrically arranged and the number of concentric layers vary (Fig. 5), whereas in the reticulate type vessels are loosely arranged forming an anastamosing system (Fig. 4).

The arrangement of vessels in the gall is quite different from that of the normal stem where the vessels of the secondary xylem is vertically arranged. Vessel arrangements in the knob gall is similar to that of the floral gall in *Pongamia* reported earlier by Govindrajalu and Lourduswamy (1980). Proliferation of the parenchymatous cells with the differentiation of the vasculature and its further rearrangement into different forms (concentric and reticulate) leads to the formation of the gall, which is in agreement with Hough's (1953) conclusion that the gall is a result of both differentiation and redifferentiation of which the latter is a major phenomenon.

Biochemical studies

To characterise the biochemical basis of such flowery galls, some enzymes involved in auxin catabolism and phenolic contents were determined (Table I).

IAA oxidase activity could be detected neither in crude extract nor when the extract was clarified by sephadex G-25 filtration. This indicated that oxidation of IAA was prevented causing hyper-auxinity in gall tissue and such a relationship between hyper-auxinity and tumorigenesis has been reported in various forms of plant galls (Stonier, 1972; Tandon and Arya, 1980; Wegen and Glase, 1981). Polyphenoloxidase catalyses the conversion of monophenols to dihydroxy phenols and to quinones, the latter being inhibitors of IAA oxidase. The polyphenoloxidase activity increased with progressive stages of gall formation in cocoa. The total and o-dihydric phenols decreased with increase in polyphenoloxidase activity contrary to earlier observations (Tandon and Arya, 1982; Wegen and Glase, 1981). It is probable that the endogenous level of phenolics are not limiting in the process of gall formation.

The activity of peroxidase increased as the gall formation progressed as

FIGS. 1—5. CUSHION GALLS OF COCOA

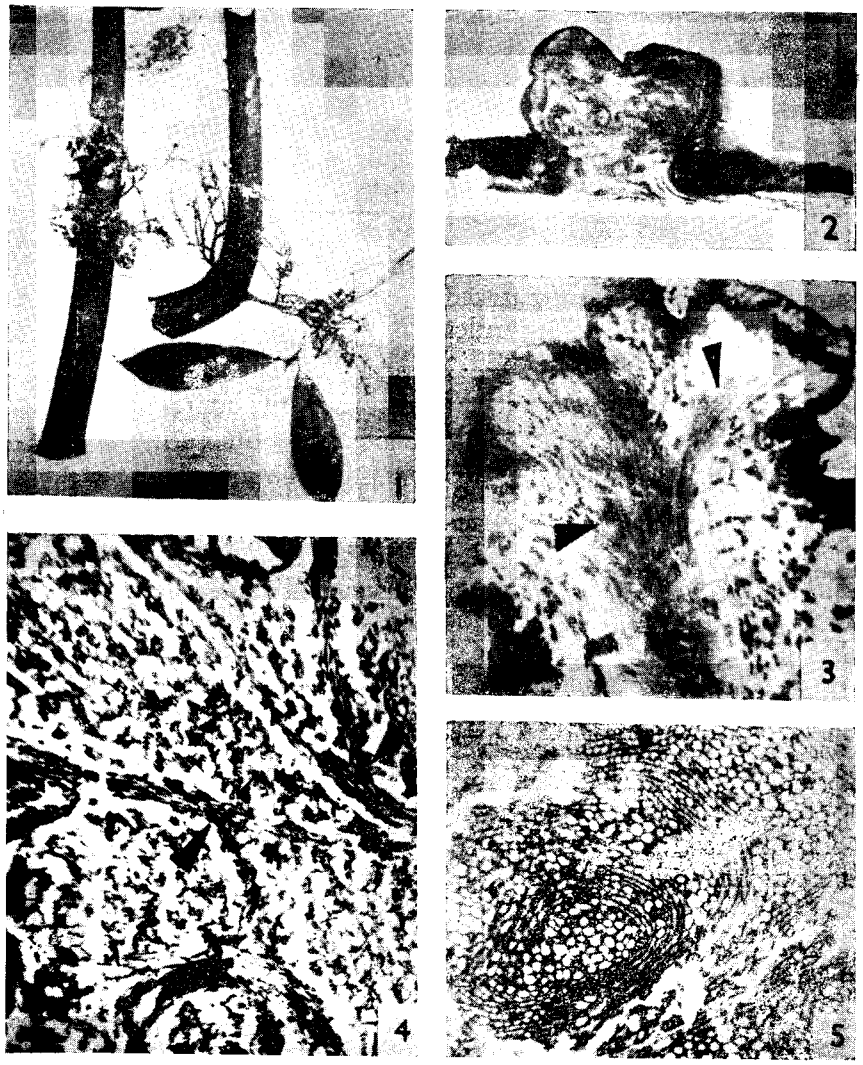

Fig. 1. Cocoa stem with fan galls; Fig. 2. A longitudinally cut knob gall; Fig. 3. L.S. of a young knob gall with protuberances and definite orientation of the vasculature (arrows); Fig. 4. L.S. of a well developed knob gall showing reticulate arrangement of the xylary vessels; Fig. 5. L.S. of a well developed knob gall showing concentric arrangement of the xylary vessels

Table I. *Activities of peroxidase, polyphenoloxidase and content of phenols*

Tissue	Peroxidase $\triangle A_{485}$ min^{-1} g^{-1}	Polyphenol oxidase $\triangle A_{495}$ min^{-1} g^{-1}	O-dihydric phenol mg g^{-1} fresh wt.	Total phenol mg g^{-1} fresh wt.
Healthy	0.60	-	1.64	18.20
Apparently healthy	0.80	0.015	2.24	13.00
Diseased				
Initial	1.30	0.043	1.16	12.80
Mild	0.90	0.040	1.08	13.80
Advanced	1.50	0.041	0.80	10.40

compared to healthy stem tissue. The gall tissues did not show marked differences in lignification which rules out the involvement of peroxidase in lignification. It is rather difficult to explain the increase in peroxidase based on the present data. These results point out that hyperauxinity might be one of the causes for gall formation. A corroborative evidence for this is provided with the effect of some growth regulatory substances in green-point gall of cocoa (Mitchell, Hutchins and Marth, 1965) where 2, 3, 5-triodobenzoic acid applied to apical portion of stem increased branching in galls. The auxin levels would become higher as TIBA inhibits polar transport of auxins. However, the causal agent for such abnormal growth of cushion remains to be determined.

Cushion galls of one or the other type have been reported from most of the cocoa growing countries (Thorold, 1975). Though occurrence of cushion galls remained unimportant for many years, of late, severe incidence of the disorder in some locations is being reported. In some of the areas in Western Nicaragua, Eastern and Central Costa Rica and Northern Columbia more than 90 per cent of the trees in a few cocoa gardens were reported to be affected and it is stated that this disorder may assume alarming proportions in areas as yet mildly affected.

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