reported[1-7]. Thus the use use of phenylpropiolic acid for the synthesis of 4-phenylcoumarins appeared to be promising though the yields are low and a new synthesis of methyldalbergin has thus been achieved.

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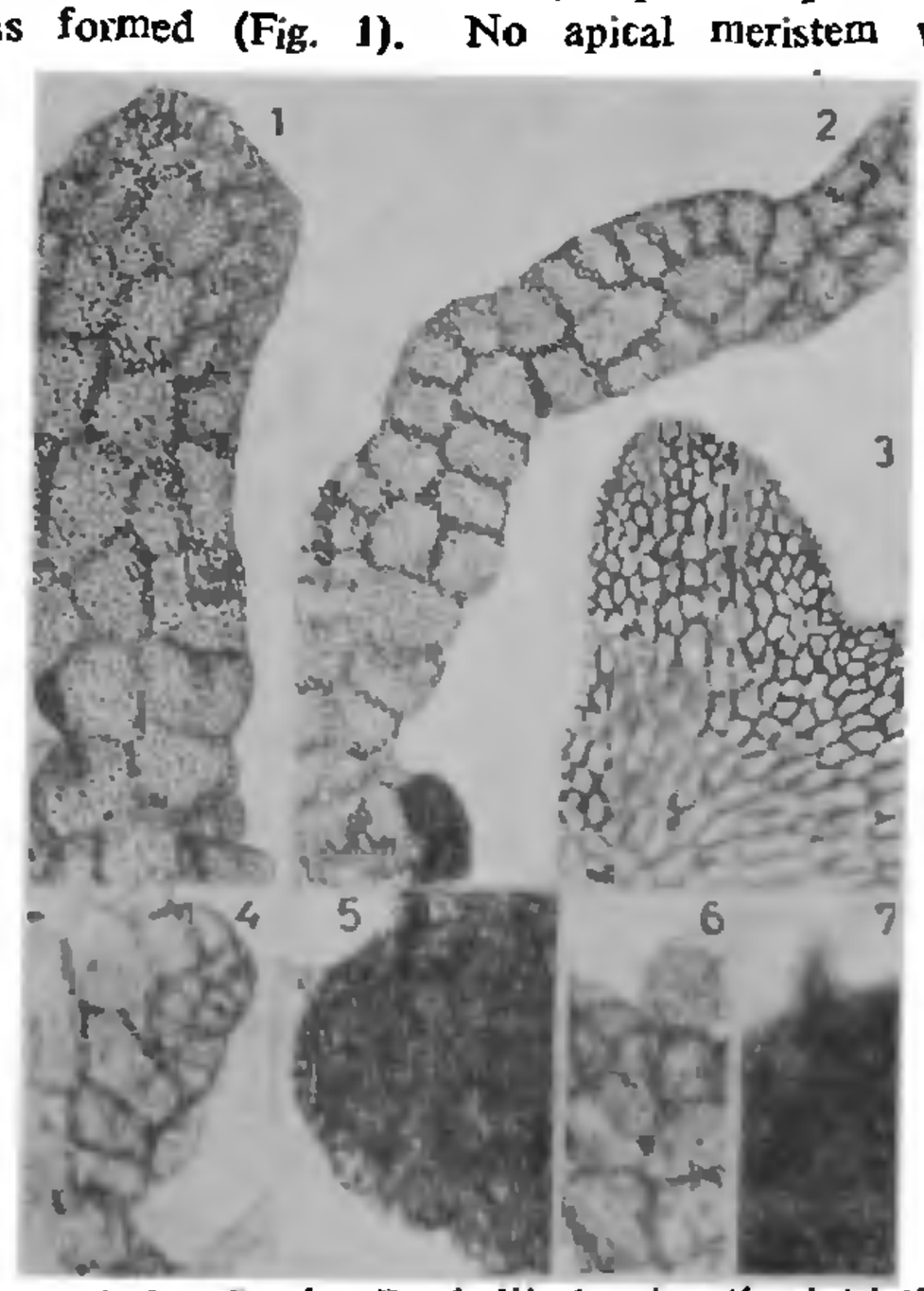
IN VITRO MORPHOGENETIC STUDIES ON THE GAMETOPHYTE OF *ANOGRAMMA LEPTOPHYLLA* (SW.) LINK.

AFTER the first publication of morphological description of the gametophyte of *Anogramma lepotophylla* by Goebel[2], no detailed information is available regarding its development, formation of tubers and sex organs[3]. The gametophyte, being perennial, bears a striking resemblance with that of a bryophyte plant rather than a short-lived gametophyte of other homosporous ferns[4]. Secondly, the growth is predominantly lopsided; and the axiate growth pattern, which characterizes the fern gametophyte in general[1], is deranged, because of the unique nature of this gametophyte, it was felt desirable to see how far the above-mentioned deviations affect the pattern of prothallial development, formation of tubers and sex organs of this interesting plant.

The spores were collected from Mussoorie in late September and surface sterilized in 2% aqueous calcium hypochlorite for 8–10 minutes and sown on Knop's medium of mineral salts, 10 mg/l ferric citrate and 1 ml/l Nitsch trace elements gelled with 0·8% agar. The cultures were maintained under 12 hr. photoperiod at 25 ± 2° C.

Development of the Prothallus

The spores germinated five days after inoculation. The papillate germ cell emerged after rupturing the spore wall, and underwent first mitotic division resulting in two highly unequal cells, the smaller rhizoidal initial and the larger prothallial initial. The rhizoidal initial developed into a rhizoid and the prothallial initial underwent successive transverse divisions to give rise to a uniseriate filament upto five cells long. But sometimes in 2-celled filaments, a vertical division was laid in its terminal cell and the two resultant cells underwent further transverse divisions to give rise to V-shaped twin protonemata. In yet other cases, the spore cells divided by a vertical wall and gave rise to two almost equal-sized cells; the two resultant cells gave rise to a filament each. In such cases the first rhizoidal cell was dealyed since it differentiated after a transverse division in one of the two cells of V-shaped protonemata. The two branches of the V-shaped protonema sometimes grew equally or in other cases, one of the branches over-topped the other. Thus unlike most of the homosporous fern gametophytes, the pattern of protonemal development was variable. A vertical division in the terminal cell of the filamentous protonema initiated two-dimensional growth. After a short period of transverse and vertical divisions, a spatulate prothallus was formed (Fig. 1). No apical meristem was



FIGS. 1–7. Fig. 1. Prothalli showing the initiation of two-dimensional growth in the absence of a distinct meristem, × 138. Fig. 2. Irregularly-shaped prothalli, × 138. Fig. 3. A part of mature gametophyte showing diffused meristematic activity, × 70. Figs. 4–5. Initiation and subsequent developmental stages of tubers, × 160. Figs. 6–7. Formation of antheridia in the proximal region (Fig. 6) and of archegonia in the distal part (Fig. 7) of the tuber, × 160.

involved in the formation of the spatulate prothallus. In some cases the growth was very irregular and such gametophytes differentiated more than one marginal meristems (Fig. 2). As a result of meristematic activity of marginal meristems at different loci, a large number of gametophytes became lobed structures before the onset of tuber formation. As shown in Fig. 3, the mature gametophyte was not cordate, there being no distinct apical meristem.

Formation of Tubers and Sex Organs

Tubers were consistently observed in all the cultures and served three purposes (i) perennation, (ii) vegetative reproduction and (iii) production of sex organs. One or more tubers could develop anywhere from the marginal cells of the gametophyte; the site of inception was demarcated by the differentiation of a large number of rhizoids around it. The marginal cells near the rhizoids divided in different planes (Fig. 4) and gave rise to radially symmetrical tubers (Fig. 5). Three-dimensional growth was, therefore, restricted to the formation of tubers; thus unlike other homosporous ferns, no central cushion was formed within the expanded portion of the gametophytes.

As regards the sites of sex organs, the antheridial formation was restricted to the proximal region of the tubers (Fig. 6). Occasionally they were observed on the surface of the gametophyte as well in close proximity of the tuber. Archegonia were present in the distal region of the tuber (Fig. 7). Such archegonia bearing tuber has earlier been described under the term "archegoniophore" by Goebel[2].

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PEROXIDASE, PHENOLOXIDASE AND TOTAL PHENOLS IN THE SPINACH LEAVES INFECTED WITH *FUSARIUM EQUISETI*

THE fungus *Fusarium equiseti* (Corda) Sacc. causes leaf spot disease in spinach (*Spinacia oleracea* L.). Young immature leaves (1 week old) do not develop lesions but only pinkish spots are formed due to infection. The mature leaves (2 weeks old) develop circular spots of about 0·5 cm diameter with a pinkish halo. Much older (4 weeks old) leaves which turned yellow at their senescent stage were severely damaged. Only mature and older leaves show a sunken lesion area of dead tissue. Oxidative enzymes are known to play a role in the defence mechanism of plant tissues[1–3]. In view of this, the activities of peroxidase, phenoloxidase and total phenolic contents of spinach leaves under different stages of infection with *F. equiseti* are presented in this communication.

The leaf tissues from the healthy or infected areas avoiding dead tissue in the sunken lesion spot, were cut and put into chilled extractants. The fresh weight of each tissue was determined and separately homogenized in phosphate buffer (0·1 M) at pH 6·8 using a Virtis tissue homogenizer for 2 minutes at the maximum speed. The homogenate was centrifuged at 2,000 g at 4° C. The supernatant was made up to 25 ml with distilled water and used as enzyme source.

For peroxidase (E.C. 1.11.1.7) assay[4] 3 ml of 0·05 M guaiacol and 0·1 ml of tissue extract were taken in the colorimeter tube and the absorbance was adjusted to zero at 470 nm. Then 0·5 ml of 1% H_2O_2 was added to the tube and the contents were quickly mixed. Change in absorbance was recorded every 10 seconds for a period of 3 minutes. In the phenoloxidase (E.C. 1.10.3.1) assay[5] 2 ml tissue extract and 3 ml of phosphate buffer (0·1 M; pH 6·0) were taken in a colorimeter tube, and its absorbance was adjusted to zero at 495 mm. Then 1 ml of 0·01 M catechol was added, contents were mixed and change in the absorbance was recorded every 10 seconds up to 3 minutes. Heated enzyme served as control and the activity of the enzymes was expressed as increase in absorbance ΔA/minute/2 mg fresh weight of leaf tissue. For the study of total phenols tissue slices were extracted in boiling 80% ethanol (1 g/10 ml), and estimation was made with Folin-ciocaulteu reagent using catechol as the standard[5].

There was nearly four fold increase in peroxidase and phenoloxidase activities in the immature leaves in the infected areas (Table I). The increase of enzyme activities in the immature leaves was, however, three-fourths due to infection, while the leaves at their senescent stage showed no appreciable increase. Though relatively little is known regarding the biological function of peroxidase[6], its direct participation in the inhibition of fungal growth cannot be ruled out[7,8]. These enzymes bring about the oxidation of phenolic substances and the oxidation products could be toxic to the pathogen[9], especially in younger leaves. There was also an age-dependent correlation in the increase in the total phenolic content after infection and inhibition of the pathogen. Some of the phenolic contents may serve as suitable substrates for phenoloxidase which is presumed to play a role in the defence mechanism[10]. The present investigation shows a rough correlation between enzyme activities and lesion size[11], thus implicating these factors in symptom expression[12].