Positive reaction for Liebermann–Buchard test indicating the presence of triterpenoids/steroids is obtained for *S. racemosa*, *S. thyrsioides* and *S. zeylanica* as in *E. acaulis*[7]. While *S. thyrsioides* and *S. zeylanica* show positive reaction for salkowski test (steroids) and *E. acaulis*[7] for Nollers test (triterpenoids), *S. racemosa* shows positive reactions for both the tests. The other species of *Staurogyne* and *Nelsonia campestris*[7] show negative reactions for the same. Maule test is positive in *S. racemosa*, *S. thyrsioides* and *S. zeylanica* as in *Nelsonia*[7] and negative in the rest. *S. racemosa* and *S. thyrsioides* resemble *Elytraria*[7] in the presence of alkaloids.

However, some of the species of *Staurogyne* stand apart from rest of the Nelsonoideae in the doubtfully positive reaction for syringin test (*S. angustifolia* and *S. zeylanica*) as evidenced by the development of green colour in the wood, in the presence of leucoanthocyanins (*S. racemosa*) and tannins (*S. racemosa*, *S. thyrsioides* and *S. zeylanica*).

Staurogyne (present study) *Elytraria* and *Nelsonia*[7], closely resemble one another and also resemble Acanthaceae rather than Rhinantheae/Scrophulariaceae[5] in chemical characters. Besides the significant morphological features, the previous[5,7] and the present chemotaxonomic data and the data from other disciplines such as cytology, embryology, floral anatomy, foliar anatomy, palynology and stomatal ontogeny justify the retention of Nelsonioideae in Acanthaceae and negate the view of transfer to the vicinity of Rhinantheae of Scrophulariaceae as suggested by Bremekamp.[2–4] and his supporters.

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PRODUCTION OF PHENOLICS IN *ACROSTICHUM AUREUM* L. DURING GROWTH *IN VITRO*

M. A. Padhya, P. B. Kavi Kishore and A. R. Mehta

Tissue Culture Laboratory
Department of Botany
The M.S. University of Baroda
Baroda 390 002, India

Introduction

Phenolic substances, the active products of cellular metabolism, are of great commercial importance. Besides being precursors for lignin biosynthesis, they have been regarded as contributors to disease resistance (Cruickshank and Perrin[4]). Phenolic compounds have also been implicated in plant—plant alleopathic interactions. Their formation in chloroplasts, active catabolism (Barz and Hosel[1]) and high reactivity in various biological systems *in vitro* (McClure[8]) reveal the importance of polyphenols in plant life. The presence of phenolic compounds *in vivo* ferns has been reported by Bohm and Tryon[3] and Bohm[2].

The present investigation deals with the estimation of phenolics during various developmental stages of *Acrostichum aureum* L. grown *in vitro* from the spores.

Materials and Methods

Spores were surface sterilized with 5% sodium hypochlorite washed with sterile distilled water and inoculated on Knudson's basal medium as modified by Steeves *et al*[8]. The culture flasks were incubated in continuous light at 26 ± 2° C. After every 4 weeks, the gametophytes were transferred on the freshly made medium in absence or presence of (2%) sucrose. After incubation for 8 weeks, the gametophytes produced sex organs. At this stage they were flooded with sterile distilled water to ensure fertilization. The sporophytes were thus raised sexually and were grown on (2%) sucrose medium in continuous light at 26 ± 2° C. The sporophytes were also induced apogamously on high sucrose (4%) medium (Kshirsagar and Mehta[5]). Gametophytes and sporophytes were harvested every four weeks, dried and polyphenols were extracted and estimated by Folin method of Swain and Hills[9]. The standard curve was prepared using chlorogenic acid and all the phenolic contents were expressed in mg per 100 mg of tissue dry weight.

Results and Discussion

Total polyphenols estimated in the dry tissue of gametophytes and sporophytes during the course of their growth in culture and at different developmental stages are presented in Table I. Clearly a close correlation was observed between the stage of development of plant and the phenolic content of the tissue. Higher level of phenolic compounds in apogamously

TABLE I

Developmental stage of plant	Kundson's basal medium ± % sucrose	Time in weeks after inoculation of spores	Total phenolics mg %
Gametophytes (vegetative)	0·0 sucrose	4	0·82
Gametophytes (reproductive)	0·0 sucrose	8	0·92
Gametophytes (reproductive)	+2% sucrose	8	1·20
Sporophytes (sexually produced)	+2% sucrose	12	1·52
Sporophytes (apogamously produced)	+4% sucrose	12	1·70
Sporophytes (sexually produced)	+2% sucrose	16	3·3
Sporophytes (sexually produced)	+2% sucrose	20	3·8
In vivo sporophytes (fruiting)	—	—	1·72

produced sporophyte, as compared to that in the sexually raised one could be due to higher sucrose level in the medium used. Increasing carbohydrate levels are found to enhance accumulation of polyphenols in the tissue cultures derived from the higher plants (Shah *et al.*[7]). The presence of phenolics in appreciable amounts in gametophytes and sporophytes of the fern suggests that they too have capacity to synthesize them and that the well-established pathways of phenolic metabolism in angiosperm and gymnosperm also function in ferns.

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IN VITRO STUDIES ON GAMETOPHYTES OF *PTERIS VITTATA* L.

M. A. PADHYA AND A. R. MEHTA
Department of Botany
The M. S. University of Baroda
Baroda 390 002, India

IN the present investigation, the influence of mineral nutrition on the induction of apogamy was investigated. Apogamy was induced in *Pteris vittata* by culturing the gametophytes on standard dose of macroelements of Knudson's medium with 4% sucrose. Decreasing the concentration of mineral salts from the culture medium prevented apogamous response. Moreover, the differentiation of the gametophytic callus was conditioned by sucrose concentration in the culture medium.

Introduction

Apogamy was experimentally induced in sexually reproducing ferns such as *Pteridium aquilinum* (Whittier[10]); *Ampelopteris prolifera* (Mehra and Sulklyan[5]) and *Acrostichum aureum* (Kshirsagar and Mehta[1]). Manton[3] suggested that the ease with which apogamy can be induced in ferns might be due to their higher chromosome number coupled with genetic imbalance brought about by hybridization. The apogamous structures produced were mostly leaves or complete sporelings. Moreover, reduction in mineral nutrition of the prothalli of *Osmunda cinnamomea* (Whittier and Steeves[11]) and *Pteridium aquilinum* (Treanor and Whittier[9]) prevented apogamous sporophyte formation.

In the present studies an attempt was made to determine the role of mineral nutrition in the induction of apogamy in *Pteris vittata* prothalli. The capacity of the gametophytic callus for differentiation was examined.

Materials and Methods

The spores of *Pteris vittata* L. were sterilized with 5% sodium hypochlorite and inoculated on Knudson's