MICROSPOROGENESIS AND MALE GAMETOPHYTE IN THREE SPECIES OF *SETARIA* (POACEAE)

THE present investigation deals with microsporogenesis and the male gametophyte in three forage grasses, i.e., *Setaria glauca* (L.) P. Beauv., *S. intermedia* (Roxb.) Kunth and *S. verticillata* (L.) P. Beauv., of the tribe Paniceae of the subfamily Panicoideae.

The anthers are tetrasporangiate. The anther wall consists of four layers, *i.e.*, epidermis, endothecial layer, a single middle layer and the tapetum. An anther wall composed of four layers of cells seems to be a characteristic feature of the family Poaceae[1–6,13–19,23,24]. The cells of the epidermis become stretched in mature anthers and the endothecial cells develop fibrous thickenings. The middle layer is ephemeral and its cells start disintegrating at the onset of the meiotic division in the MMC. The tapetum is of the glandular type like in other grasses[1–24]. The tapetal cells remain uninucleate in *S. glauca* and *S. intermedia* but in *S. verticillata* some of the cells are occasionally binucleate. In majority of species investigated so far the tapetal cells become binucleate while they remain uninucleate in *Pennisetum typhoideum*[14], *Panicum miliare*[16] and *Eleusine compressa*[9].

Meiosis in MMC is usually normal. The microspore tetrads are isobilateral in *Setaria glauca* and *S. intermedia*. In *S. verticillata*, however, the tetrads may be isobilatral, decussate, linear or T-shaped and in still others, oblique wall formation occurs on completion of Meiosis II in one of the dyad cells. Cytokinesis is of the successive type in Poaceae excepting one or two instances[1,3–23]. Artschwager and McGuire[2] reported simultaneous cytokinesis in *Sorghum vulgare*. In *Themeda australis*, Woodland[24] occasionally noticed simultaneous cytokinesis. In the Poaceae the microspore tetrads are usually isobilateral or decussate and very rarely tetrahedral. As in *Setaria verticillata*, the formation of linear, T-shaped, decussate tetrads or oblique wall formation in one or both the dyad cells besides the isobilateral tetrads has been reported in *Eleusine coracana*[13], *E. africana*[8], *Echinochloa frumentacea*[17], *E. stagnina*[12], *Hordeum hexastichon*[22], *Stipa ischu*[7], and *Zizania aquatica*[23].

The microspores separate from the tetrad and become more or less spherical and increase in size. A vacuole appears in its cytoplasm and the nucleus moves to a peripheral position near the wall. It undergoes a mitotic division to form a small lenticular generative cell and a large vegetative cell. The generative cell moves into the cytoplasm of the vegetative cell and divides further to form two male gametes which are spherical or ovoid in shape. The vegetative nucleus shows signs of degeneration at this stage. The pollen grains are thus shed at the three nucleate stage like most other members of the family. In *Pennisetum typhoideum*, Rangaswami[20] reported that the pollen is shed in a uninucleate condition, which, however, needs confirmation. The pollen grains are monocolpate with a thick smooth exine and thin intine.

Department of Botany,
Panjab University,
Chandigarh 160 014,
India, *December* 27, 1979.

RAVINDER K. BHANWRA.
SANJIV KUMAR.
S. P. CHODA.

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SEXUALITY OF *GANODERMA COLOSSUM* (FR.) TORREND

ACCORDING to modern mycologists[3,4,6], fungi showing different types of sexuality cannot belong in the same genus. The type of mating system of a large number of species belonging to different genera of Polyporaceae have already been determined but very few

TABLE I

Pairings of 20 monosporous mycelia derived from a single sporophore of Ganoderma colossum (Fr.) *Torrend*

		A_1B_1					A_2B_2			A_1B_2				A_2B_1							
		1	3	6	7	12	2	9	14	8	10	22	24	5	15	16	18	20	21	23	25
A_1B_1	1	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	3	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	6	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	7	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	12	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
A_2B_2	2	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	14	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
A_1B_2	8	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	10	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	22	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
	24	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+
A_2B_1	5	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	15	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	16	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	18	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	20	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	21	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	23	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
	25	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−

members[1,2] of the genus *Ganoderma* (Leyss.) Karst. have been studied so far from this point of view. The present paper communicates the result of interfertility study of *Ganoderma colossum* (Fr.) Torrend a wood-inhabiting polypore of India.

Following the usual dilution method twenty-five monsporous cultures were isolated from the spores of a sporophore of *G. colossum* collected from Bankura, West Bengal, India, where it was found growing on a dead wood of *Ficus religiosa* L. The sporophore has been dried and deposited in the Mycological Herbarium of the Visva-Bharati University, under the number VBMH 79411. When each of the 25 monosporous cultures showed good growth they were checked carefully for clamp connections. The absence of clamp connections was taken as confirmation of their monokaryotic nature. Finally 20 monokaryotic cultures were taken into consideration and the inocula from these single spore cultures were placed in pairs, about 25 mm apart on 2·5% malt agar slants. The monosporous cultures were mated among themselves in this way in all possible combinations. The culture tubes containing paired inocula were incubated at room-temperature (28 ± 2° C) for about a fortnight and then the hyphae from the line of contact between the paired mycelia were examined for clamp connections.

The result of matings has been presented in Table I where a plus sign (+) indicates the formation of hyphae bearing clamp connections, and a minus sign (−) their absence.

It will be evident from Table I that the single spore cultures from one sporophore of *G. colossum* fall into four groups on the basis of their ability to form dikaryotic mycelia, recognizable by the presence of clamp connections. The genetic constitutions of the four groups have been designated as A_1B_1, A_2B_2, A_1B_2 and A_2B_1 following Nobles, Macrae and Tomlin[5]. Dikaryotic mycelia were formed only in matings between $A_1B_1 \times A_2B_2$ and $A_1B_2 \times A_2B_1$, *i.e.*, between mycelia having no common allele. Therefore, *G. colossum* possesses tetrapolar type of sexuality with allelomorphs for heterothallism at two loci.

It may be mentioned that G. colossum shows similarity in this respect with two other species of *Ganoderma*, namely, *G. appalanatum* (Pers. ex Wallr.) Pat[1], and *G. lucidum* (Leys. ex Fr.) Karst[3]. which also possess tetrapolar type of sexuality.

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Department of Botany,
Visva Bharati University,
Santiniketan 731 235 (W.B.),
February 26, 1980.

A. B. DE.

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PHYSOSTIGMINE INHIBITION OF CHOLINESTERASE ACTIVITY IN THE VENTRAL NERVE CORD OF SCORPION

It is reported earlier that the enzyme, Cholinesterase (ChE), found in the nervous system and the innervated organs of the scorpion, is inhibited by physostigmine and the effect is maximum at 1×10^{-5} M concentration[1] as is typical of true cholinesterases[2]. The present paper reports further results on physostigmine inhibition of ChE activity in the ventral nerve cord of the scorpion, *Heterometrus fulvipes* C. Koch.

After adapting the animals to the laboratory conditions[1] they were dissected and the ventral nerve cords were isolated into cold scorpion ringer[3]. The enzyme activity was determined, using the method of Metcalf as outlined by Augustinsson[2] with suitable modifications[1], under different experimental conditions. The incubation mixture contained 0·1 ml of 1% homogenate (W/V) in 0·25 M sucrose soln. and 1·0 ml of buffer substrate soln (9 vols. of 7·2 pH phosphate buffer + 1 vol. of 0·04 M acetylcholine chloride soln.). The mixture was incubated at 37° C for ½ hr and the reaction was stopped by adding 2·0 ml of alkaline hydroxylamine hydrochloride soln. and 1·0 ml of 1 : 1 hydrochloric acid soln. The colour developed by the addition of 1·0 ml of 10% ferric chloride soln. was read at 540 nm. The amount of unreacted acetylcholine was determined from the standard graph and the enzyme activity was expressed as μmoles acetylcholine hydrolysed/gm wet wt. of tissue/hr. The difference in the enzyme activity levels in the presence and absence of physostigmine was calculated and expressed as per cent inhibition of the enzyme activity.

Isolated nerve cords were exposed to 1×10^{-5} M physostigmine for different periods of time and the enzyme activity determined. The activity decreased by 35% within 10 min exposure and an hour's exposure inhibited the activity by 80%. Thus the magnitude of the inhibitory effect increased with period of exposure (Fig. 1 A).

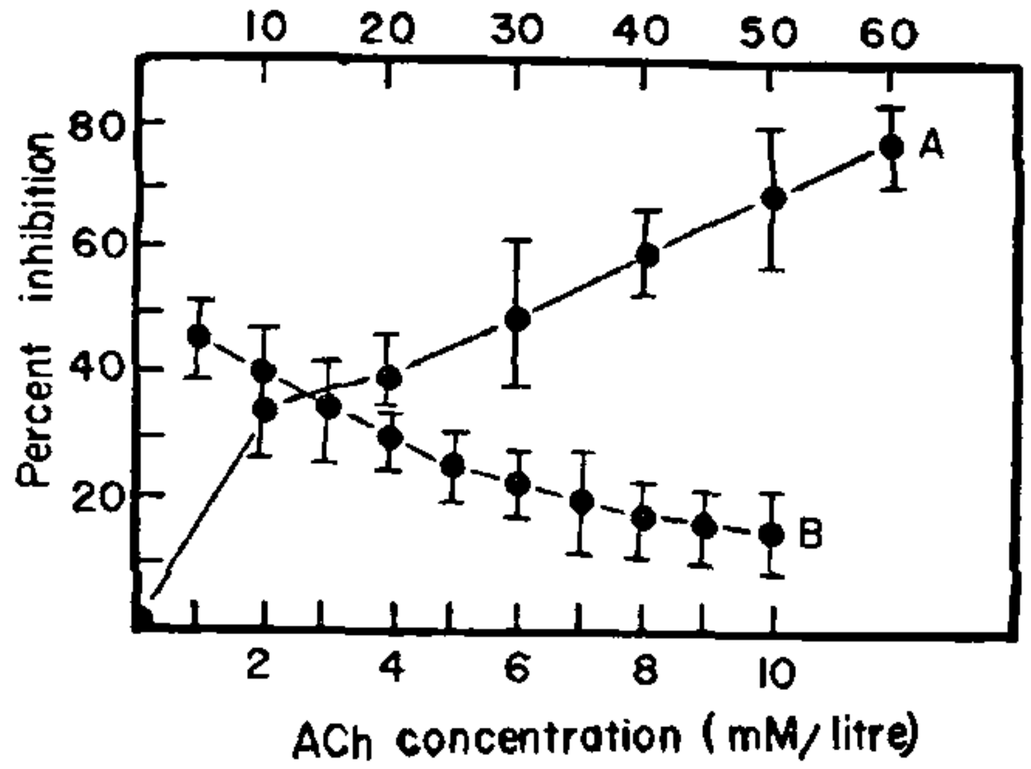

FIG. 1. Physostigmine inhibition of ChE activity in relation to period of exposure (A) and substrate (ACh) concentration (B).

The inhibitory effect was also studied in presence of different concentrations of the substrate, acetylcholine (ACh). Inhibition was seen at all substrate concentrations but decreased with an increase in the latter and hence the two were inversely related (Fig. 1 B). Lineweaver–Burke plots showed that V_{max} is not altered in presence of physostigmine but K_m decreased from $1{\cdot}2 \times 10^{-4}$ M to $3{\cdot}8 \times 10^{-4}$ M. Physostigmine seemed to lower the affinity of the enzyme molecule to the substrate without altering the maximal velocity and thus act as a competitive inhibitor. Effective inhibition of ChE at low substrate concentrations and its decrease at higher substrate concentrations also suggests the same. Competitive inhibitory action of physostigmine on ChE is known in several cases[4] including the ChE of the scorpion heart muscle[5] but its action on ChE of squid ganglion is, however, shown to noncompetitive[6]

ChE activity in the ventral nerve cord of scorpion shows a regular diurnal rhythm with maximum at 16·00 hr and minimum at 4·00 hr[7]. In order to see whether there is any such dirurnal variation in ChE inhibition by physostigmine, the effect was studied at different times of the day by adding 0·5 ml of the drug to the incubation mixture besides the other reagents. It was evident that physostigmine inhibition also showed a diurnal rhythm but it was minimum at 16·00 hr and maximum at 4·00 hr (Fig. 2).