EFFECT OF ACETOHYDROXAMIC ACID AND *p*-HYDROXYMERCURIBENZOATE ON UREASE ACTIVITY OF BLUE-GREEN ALGAE

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THE chelating agent acetohydroxamic acid[1] and the sulphhydryl reagent, *p*-hydroxymercuribenzoate[2] inhibit the enzyme urease. The inhibition of jack bean urease by acetohydroxamic acid is due to its reversible binding to nickel ions at the active site[3]. *p*-Hydroxymercuribenzoate binds to the sulphhydryl (−SH) groups which are in some way involved in the action of the enzyme[4, 5]. These agents have not been tested on urease from blue-green algae, except for a single report[6]. The present paper describes the effect of acetohydroxamic acid and *p*-hydroxymercuribenzoate on urease from the blue-green algae *Anabaena doliolum* and *Anacystis nidulans*.

Anabaena doliolum Bharadwaja and *Anacystis nidulans* IU 625 (ATCC 27144) were grown in modified Chu No. 10 medium[7] at 24±1°C and illuminated with daylight fluorescent tubes (intensity 2500 lux at the surface of the vessels) for 14 h/day. Cell-free extracts were prepared by grinding mid-logarithmic-phase cells in a prechilled glass mortar and pestle at 4°C in an ice bath with an equal volume of acid-washed sand. Crude enzyme was extracted with 5 mM phosphate buffer (pH 7). The supernatant collected after centrifugation was used for the estimation of urease activity. The enzyme extract was incubated with different concentrations of acetohydroxamic acid and *p*-hydroxymercuribenzoate at 37°C for 10 min in 5 mM phosphate buffer (pH 7). The reaction was initiated by the addition of urea (2 μmol in a final volume of 2 ml of 5 mM phosphate buffer, pH 7), and terminated by the addition of 4 ml of mixed reagent used for the colorimetric assay of urea[8]. Protein was estimated by the method of Lowry *et al*[9] with bovine serum albumin as the standard.

Figure 1 shows that both *Anabaena* and *Anacystis* ureases were inhibited by acetohydroxamic acid and *p*-hydroxymercuribenzoate; however, the latter reagent appears to be a more powerful inhibitor. *p*-Hydroxymercuribenzoate at 1.5 μM concentration reduced enzyme activity by 50% (figure 1A), whereas, for the same level of urease inhibition, acetohydroxamic acid was required at 3 μM (figure 1B). Complete inhibition of urease was observed at

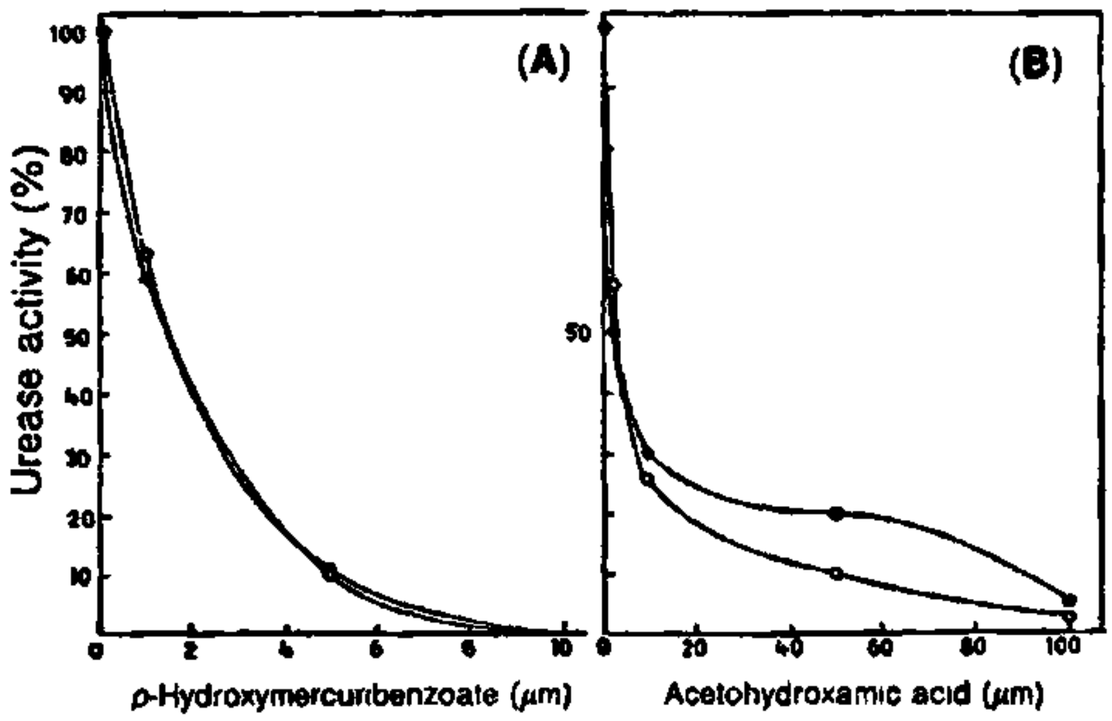

Figure 1A, B. Effect of *p*-hydroxymercuribenzoate (**A**) and acetohydroxamic acid (**B**) on urease activity of *Anabaena doliolum* (■———■) and *Anacystis nidulans* (□———□). Urease activity of 100% corresponds to 495.5 and 450 nmol urea hydrolysed/mg protein/h for *Anabaena doliolum* and *Anacystis nidulans* respectively.

10 μM *p*-hydroxymercuribenzoate, but was not attained even at 100 μM acetohydroxamic acid. Residual urease activity, 5% and 2.5% respectively in the extracts of *Anabaena doliolum* and *Anacystis nidulans*, was observed even at 100 μM acetohydroxamic acid. Interestingly, the inhibition curve is steep up to 10 μm acetohydroxamic acid and flatter beyond this concentration. The inhibition of urease by acetohydroxamic acid suggests that nickel is an essential component of the enzyme while the inhibition by *p*-hydroxymercuribenzoate indicates the involvement of thiol groups in enzyme activity.

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HISTOLOGICAL STUDIES OF MICROSPOROGENESIS AND MALE GAMETOPHYTE DEVELOPMENT IN AMBRETTE (*ABELMOSCHUS MOSHATUS* MEDIC.)

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ABELMOSCHUS MOSCHATUS Medic. (Malvaceae) is an important indigenous aromatic plant. The volatile Ambrette oil in the seed coat is noted for its rich, sweet floral musky, distinctly wine-like odour with a boquet of roundness, rarely found in any other perfumery material and a tenacity of odour which is almost incredible[1].

Although the crop is of great economic importance, very little work on this crop has been attempted. The present work reports histological studies of the microsporogenesis in Ambrette. The formation and development of pollen grains have important implications to the breeder in understanding the pollen dehiscence mechanism and pollen grain viability. Hence this work was initiated to study the histology of pollen grain formation to help the breeder in formulating appropriate procedures of crop improvement.

The flower buds of all the representative stages in the development of anther were sampled and categorized into different groups based on their size. The flower bud was fixed and sectioned by a procedure described earlier[2]. Sections were stained with appropriate histochemical stains and observed under microscope. The photomicrographs were taken with the help of a Carl Zeiss photomicrograph.

The micrometric observations at various stages of anther development are presented in table 1. The cross-section of anther revealed a mass of isodiametric cells bounded by an epidermis. The archesporial cells were found initiated hypodermally. These cells divided periclinally to produce primary sporogenous cells to the interior and primary parietal cells to the exterior. Further, periclinal and anticlinal divisions differentiated into endothecium, middle layer and tapetum. The primary sporogenous cells divided mitotically and gave rise to sporogenous tissue (figure 1). At this stage pollen sac diameter was 93.03 μm. The number of sporogenous cells in the sac was 4.4; the size of individual sporocyte was 23.69 μm.

The thickness of tapetum, endothecium, middle layer and epidermis in anther was respectively 11.40, 6.16, 12.32 and 11.40 μm. With the differentiation of pollen mother cells the tapetal cells enlarged and their cytoplasm became dense. Micrometric observations showed increased pollen sac diameter (106.86 μm) and the number of PMC's was 6.29 while their size was 36.85 μm. The thickness of tapetum and endothecium increased to 12.32 and 8.8 μm, respectively. After this, there was disintegration of middle layers and the epidermis size decreased to 10.20 μm.

The pollen mother cells underwent meiosis I and II, forming isobilateral microspore tetrads. The tapetum was glandular with dense cytoplasm, remained peripheral and reduced in its size (figure 2); the pollen sac diameter increased to 193.60 μm. The number of isobilateral tetrads was 4.89 and the size

Table 1 *Micrometric observations during microsporogenesis and malegametophyte development in A. moschatus*

	Pollen sac (μm)				Thickness (μm)			
Stages	Diameter	No. of cells	Individual cell size	Nuclear size	Tapetum	Endothecium	Middle layer	Epidermis
Sporogenous tissue	93.029	4.40	23.69	—	11.40	6.16	12.32	11.40
Pollen mother cells	106.86	6.29	36.85	—	12.32	8.80	D	10.20
Tetrads	193.60	4.89	63.067	—	12.76	11.24	D	8.80
Microspores	240.60	22.00	52.80	—	18.48	14.08	D	6.16
Pollen grains	314.82	27.133	87.45	8.8	D	36.46	D	5.029

D, Disintegrated.