stages of pollen (figure 3) were derived from further divisions of 4-celled pollen which in turn proliferated into a callus mass (figures 4–5). The plantlets (30 ± 2 per explant) are produced from these calli containing embryogenic masses of cells which were oval, thin-walled and uniformly staining. The non-embryogenic cells were comparatively larger in size, irregular in shape and thick-walled.

The ploidy of the 84 randomly selected plantlets was determined, and they were then transferred to sterilized vermiculite and later established in soil. In *N. plumbaginifolia*, cytological screening of root tips of regenerants revealed that eight (9.5%) of the plantlets produced were haploids (figures 6–8). However, Tran Thanh Van and Trinh[2] reported the frequency of haploid production as 3% in the presence of IAA (10^{-6}M) and KN (10^{-6}M). Five out of eight (62.5%) of the haploid plants transferred to the soil, have survived transplantation.

Studies are in progress to develop competence in the multicelled, globular pollen embryoids for undergoing normal embryogenesis, through stages reminiscent of late zygotic embryogenesis, leading to the production of haploid plantlets without an intervening callus stage.

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1. Murashige, T. and Skoog, F., *Physiol. Plant.*, 1962, 15, 437.
2. Tran Thanh Van, K. and Trinh, T. H., *Z. Pflanzenphysiol.*, 1980, 100, 379.

LEACHING OF PHYCOBILIN PIGMENTS TO SALINITY RESPONSE IN *OSCILLATORIA SANCTA*

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In *Oscillatoria sancta* the effect of salinity (NaCl concentration) on the pigments, photosynthesis, protein content and phycobilin leaching is reported. *O. sancta* was isolated from the paddy fields of Palghat District, Kerala State, India. The culture was made axenic by antibiotic treatment[1] and maintained in BG_{11} medium[2] at 28 ± 2°C under illumination of 2000 lux units in a 12/12 hour light dark regime. For all experiments involving varying salinity, the sea water concentration of 35 g NaCl, l^{-1} was taken as 100% salinity and NaCl was added to BG_{11} medium in appropriate quantities to obtain the various concentrations (10, 40, 80 and 100%).

Chl *a* was determined[3] by measuring the optical density of the pigment extract (in 80% acetone) at 663 nm in a Bausch and Lomb Spectronic 20. Phycobilins were estimated according to Bennet and Bogarad[4]. The cells (after extraction in acetone) were suspended in 50 mM phosphate buffer and disrupted in an MSE ultrasonic disintegrator, centrifuged for 15 min at 3000 r.p.m. and the optical density of the supernatant was measured at 562, 615 and 652 nm. Leached out phycobilins from culture medium was separated by centrifugation and the phycobilin content of the supernatant was estimated. The leached out pigments were scanned in a recording Spectrophotometer (Unicam SP 800). The photosynthetic oxygen evolution of intact filaments was measured at 28°C using an oxygen electrode (Clark type YSI model 53) at a light intensity of 5000 lux units. The protein content of intact filaments and leached out pigments was determined[5] using BSA as the standard.

Figure 1 shows the effect of various concentrations of NaCl on growth (measured in terms of Chl *a*), phycobilin pigments, photosynthetic oxygen evolution and total protein in *O. sancta*. This taxon, when grown in the presence of varying salinity (NaCl concentration) levels was unable to tolerate higher concentrations (80 and 100% of sea salt concentrations). It grew well only up to 10% of sea salt concentration in the medium and may be called a microhalotolerent form[6]. Photosynthetic oxygen evolution dropped to a very low level within 48 hr in the higher concentration (80 and 100% of sea salt concentrations). The intact phycobilin pigment components (phycocyanin, phycoerythrin and allophycocyanin) and the protein were similarly affected at high salinity. The failure of the organism to grow at higher concentrations could be due to major changes that were brought about in the photosynthetic pigments and consequently photosynthesis. It is the photosynthetic apparatus that gets adapted quickly to salinity levels in halotolerant forms[7–9]. Our results suggest that in forms that do no tolerate higher salinity the photosystems probably are affected first. In *O. sancta* at higher concentrations of NaCl, the components of the accessory pigments (phycobilins) that form the major core of the light harvesting photosystem were leached out into the medium. Scanning of the leached out phycobilin pigment content showed the presence of phycocy-

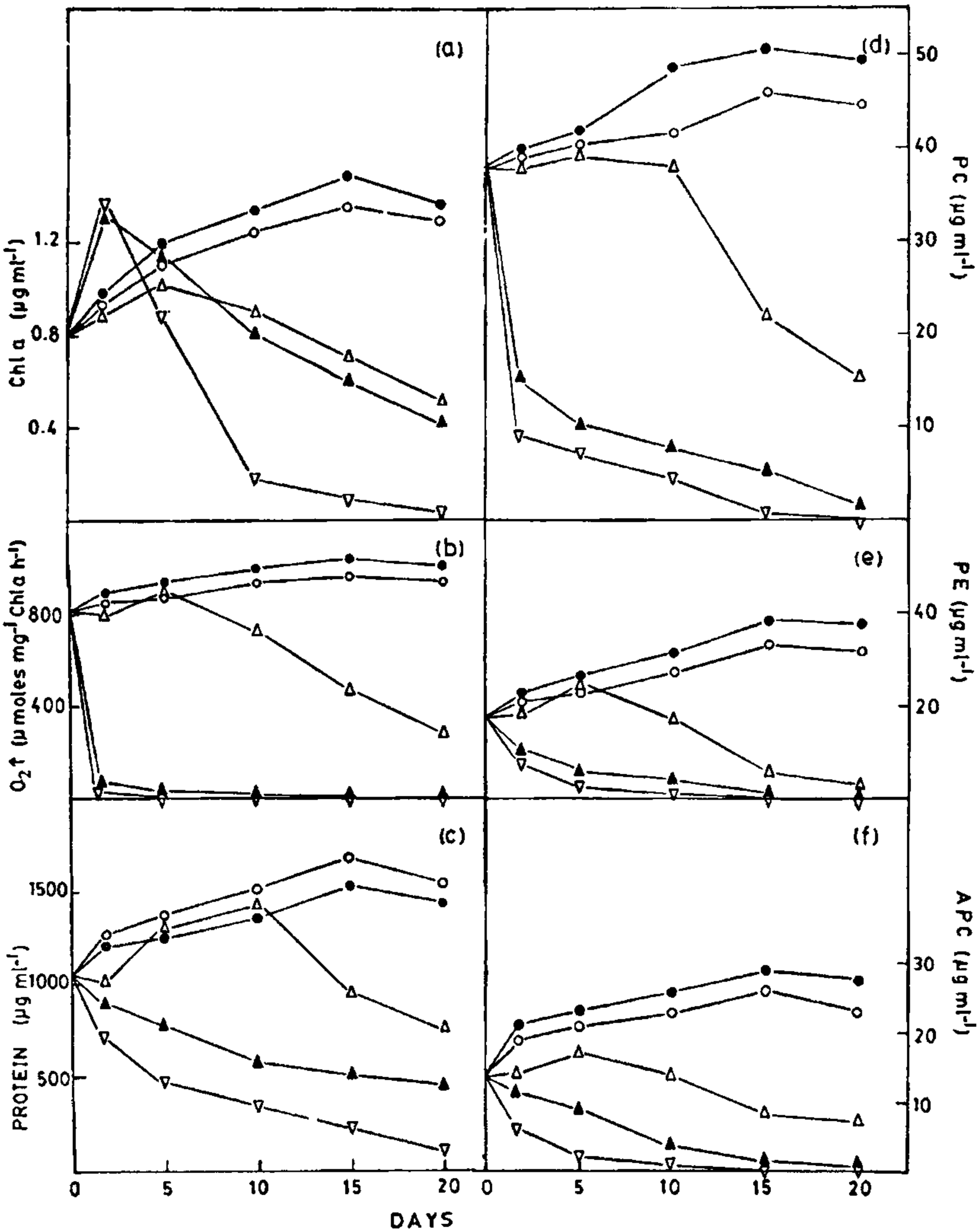

Figure 1. Effect of increasing salinity on growth rate (a) Photosynthetic oxygen evolution, (b) Proteins, (c) Phycocyanin, (d) Phycoerythrin, (e) and Allophycocyanin (APC), (f) in *O. sancta*. Control (o-o), 10% (●-●), 40% (△–△), 80% (▲–▲) and 100% (▽–▽) sea salt concentration.

anin (615 nm) and phycoerythrin (562 nm) peaks that correspond to those separated from intact cells in many related genera[10,11]. Consequently, the photosynthetic oxygen evolution dropped within 48 hr affecting further growth of the organism. Table 1 shows the leaching of phycobilin pigments and proteins (phycocyanin, phycoerythrin and allophycocyanin), when the cells were grown in low and high salinity. It may be pointed out here that Smith *et al*[12] reported severe disintegration of filament

Table 1 *Leaching of phycobilin pigments and proteins in response to salinity in O. sancta*

Sea salt concentration	Phycobilins pigments (μg/ml)	Days				
		48 hr	5	10	15	20
40%	PC	–	–	–	07.27	03.98
	PE	–	–	–	05.66	02.47
	APC	–	–	–	02.63	00.877
80%	PC	13.63	09.81	05.41	03.61	01.74
	PE	20.03	13.98	06.91	03.71	02.07
	APC	07.95	04.91	01.50	01.49	00.25
100%	PC	16.77	16.09	07.52	03.33	–
	PE	22.31	17.45	08.48	05.09	–
	APC	12.56	07.70	03.63	00.30	–
	Proteins					
40%	μg/ml	–	–	–	35	20
80%	μg/ml	400	219	76	20	06
100%	μg/ml	520	236	50	08	–

breakage, phycocyanin release and nitrogenase inhibition when *Anabaena cylindrica* was transferred to higher concentration of buffers like HEPES, Tris HCl, sodium phosphate, sodium sulphate and also NaCl.

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1. Droop, M.R., *Br. Phycol. Bull.*,1967, **3,** 295.
2. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier, R. Y., *J. Gen. Microbiol.*, 1979, **111,** 1.
3. MacKinney, G., *J. Biol. Chem.*, 1941, **140,** 315.
4. Bennet, A. and Bogorad, L., *J. Cell. Biol.*, 1973, **58,** 419.
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193,** 265.
6. Anand, N., *Curr. Sci.*, 1980, **49,** 314.
7. Tel-Or, E., *FEBS Lett.*, 1980a, **110,** 253.
8. Tel-Or, E., *Appl. Environ. Microbiol.*, 1980b, **40,** 689.
9. Tel-Or, E. and Melamed-Harel, *Photosynthesis VI, Photosynthesis and productivity, photosynthesis and environment.*, (ed.), George. Akoynoglou, Balaban International Science Service, Philadelphia, 1981, p. 455.
10. Glazer, A. N., *Annu. Rev. Microbiol.*, 1982, **36,** 173.
11. Glazer, A. N., Lundell, D. J., Yamanaka, G. and Williams, R. C., *Ann. Microbiol. (Inst. Pasteur)*, 1983, **B134,** 159.
12. Smith, G. D., Mackey, E. J. and Daday, A., *J. Gen. Microbiol.*, 1983, **129,** 3099.

SEQUENTIAL ANALYSIS OF MEIOSIS IN *GLORIOSA SUPERBA* L.

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CONTROVERSY regarding certain stages of first prophase of meiosis in plants still exists. Moens[1] observed that a synizetic knot stage precedes pachytene and there is a diffuse stage after pachytene in tomato. The diffuse stage was previously thought to be an artefact[2,3]. Since then a diffuse stage was reported in many plants[4]. A sequential analysis is possible in tomato as there is a developmental gradient in the anthers. However, in many plants it is difficult to determine precisely the sequence of stages in meiosis. In the present study meiosis was examined in diploid *Gloriosa superba* with special emphasis on prophase-I stages. *G. superba* is a favourable material for sequential analysis since there is high synchrony of cell division between six anthers in a flower bud and pollen mother cells within a single anther. By fixing anthers from the same flower bud at 1 and 2 hr time intervals it was possible to determine the exact sequence of meiotic prophase stages.