Folia Microbiol. 44 (4), 411–418 (1999) http://www.biomed.cas.cz/mbu/folia/

Viability of Dried Vegetative Trichomes, Formation of Akinetes and Heterocysts and Akinete Germination in some Blue-Green Algae under Water Stress

S.C. AGRAWAL, V. SINGH

Department of Botany, University of Allahabad, Allahabad, India

Received 19 April 1999

ABSTRACT. Almost all dried vegetative trichomes of *Anabaena iyengarii*, *Westiellopsis prolifica* and *Nostochopsis lobatus* died within 1 h, while those of *Oscillatoria acuminata* retained viability to some extent for 1 d under similar storage conditions. The viability of dried vegetative trichomes of *O. acuminata* decreased about equally on storage at 20 °C in the light or in the dark, but dropped rapidly at 12 and 0 °C in the dark. Vegetative trichomes of *A. iyengarii*, *N. lobatus* and *W. prolifica* were more sensitive to frost than those of *O. acuminata*, and this correlated with their low resistance to desiccation because both types of exposure involved osmotic stress. Both dried and wet akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* were about equally viable when stored at 20 °C in the light or the dark or at 12 and 0 °C in the dark, but their germination ability decreased on storage at 0 °C. The water stress imposed on growing vegetative trichomes either in high-agar media or in NaCl-supplemented liquid media reduced the survival of *O. acuminata* trichomes, decreased or totally suppressed akinete and heterocyst formation and akinete germination in *A. iyengarii*, *W. prolifica* and *N. lobatus*. The sensitivity decreased in the sequence *A. iyengarii* < *W. prolifica* ≈ *N. lobatus*. The akinete germination in all three blue-green algae was more sensitive to physiological water stress than their formation. In all of them, akinetes formed under water stress were equally viable as those formed under normal conditions. Trichomes of *O. acuminata* became broader when grown in 0.5–0.8 mol/L NaCl-supplemented media, probably due to polyol accumulation, and they also developed a thin sheath-like structure.

The ability of blue-green algae to withstand drying has been studied by, *e.g.*, Cameron and Blank (1966), Whitton (1987), Scherer and Zhong (1991) and Tomaselli and Giovannetti (1993). The present study examines the viability of dried vegetative trichomes, formation of akinetes and heterocysts, and akinete germination in some blue-green algae under water stress.

MATERIAL AND METHODS

The four blue-green algae used were *Oscillatoria acuminata* GOMONT, *Anabæna iyengarii* var. *tenuis* RAO, *Westiellopsis prolifica* JANET and *Nostochopsis lobatus* WOOD. Trichomes of *O. acuminata*, *W. prolifica* and *N. lobatus* were isolated from paddy fields, those of *A. iyengarii* were from a freshwater pond. Clonal cultures of *O. acuminata* were raised through hormogones, while those of *A. iyengarii*, *W. prolifica* and *N. lobatus* through germinating akinetes and were maintained in liquid BG_{11} medium (Stanier *et al.* 1971) adjusted to pH 7.5, at 22 ± 1 °C and light intensity of *ca.* 2 klx from daylight fluorescent tubes for 16 h a day.

Trichomes of *O. acuminata* were straight with briefly tapering ends (Fig. 1A), while those of *A. iyengarii* were straight or irregularly curved and had barrel-shaped vegetative cells, more or less barrel shaped heterocysts, and ellipsoidal akinetes with rounded ends in pairs on either side of a heterocyst with a smooth wall (Fig. 1C, D). *W. prolifica* trichomes were branched. The vegetative cells of their main axis were constricted at cross-walls while those in their branches were not. The akinetes of the alga were spherical, greenish yellow in color and measured 6–10 μm in diameter (Fig. 1E, F). *N. lobatus* trichomes were richly branched. They had barrel-shaped vegetative cells which differentiated into spherical yellowish green akinetes measuring about 7.5 μm in diameter (Fig. 1G, H).

About 2 % of vegetative cells of *A. iyengarii*, 1 % in *W. prolifica*, and 2 % in *N. lobatus* differentiated into heterocysts within 15 d of inoculation in liquid BG_{11} media, and these percentages did not change much thereafter. The akinete formation was observed to increase progressively from 1 to 10 % in *A. iyengarii*, from 40 to 99 % in *W. prolifica* and from 15 to 97 % in *N. lobatus* within 30–60 d of inoculation in liquid BG_{11} media. When transferred to fresh media, the akinetes harvested from old media germinated into new trichomes progressively at a rate of about 10–70 % in *A. iyengarii*, 19–82 % in *W. prolifica* and 8–65 % in *N. lobatus* within 10–25 d of inoculation.

Viability of dried vegetative trichomes and akinetes. Similarly blot-dried 7-d-old, actively growing vegetative trichomes of *O. acuminata*, *A. iyengarii*, *W. prolifica* and *N. lobatus*, and freshly formed

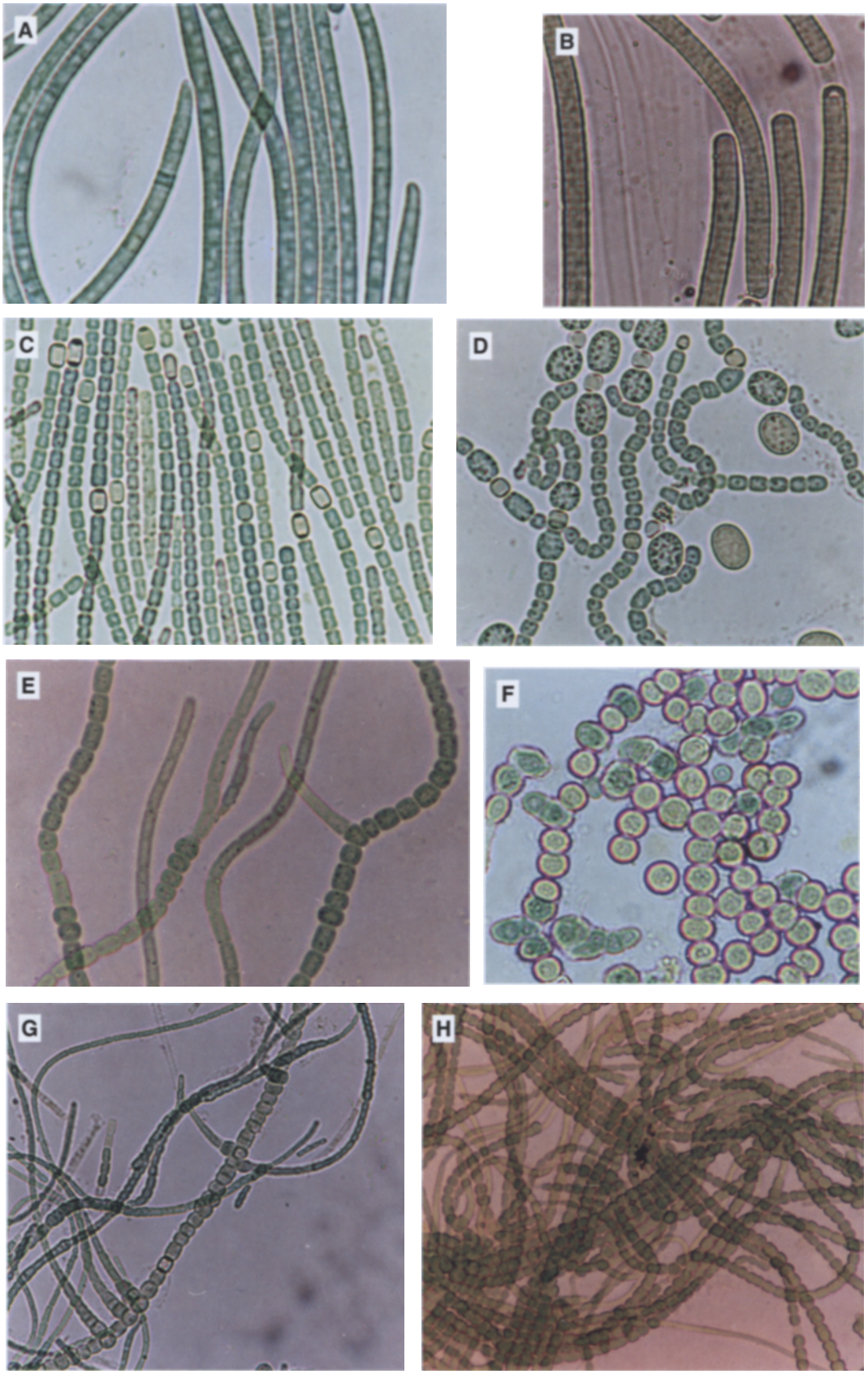

Fig. 1. **A**: *O. acuminata* trichomes, 4½ µm broad when grown in control liquid medium; **B**: *O. acuminata* trichomes, 6¾ µm broad and having a thin sheath-like structure when grown in 0.8 mol/L NaCl liquid medium; **C**: *A. iyengarii* vegetative trichomes; **D**: *A. iyengarii* akinetes bearing trichomes; **E**: *W. prolifica* trichomes; **F**: *W. prolifica* akinetes; **G**: *N. lobatus* trichome; **H**: *N. lobatus* akinetes.

akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* obtained from liquid BG_{11} media were separately placed on a filter paper and kept in desiccators over fused calcium chloride at 20 °C in the light (10 h duration at *ca.* 1.4 klx intensity), 20 °C in the dark, 12 °C in the dark and 0 °C in the dark for various time periods from 1 h to 2 d for vegetative trichomes and 1 month for akinetes. Vegetative trichomes and akinetes suspended in distilled water under similar storage conditions served as controls. The viability of dried vegetative trichomes was determined by counting the number of surviving vegetative trichomes after 15 d of inoculation in liquid BG_{11} media under normal culture conditions while the akinetes count was estimated by measuring the percentage of germinated akinete 15 d after inoculation in liquid BG_{11} media under normal culture conditions.

Survival of vegetative trichomes of O. acuminata *and formation of akinetes and heterocysts in other algae under water stress*. Seven-d-old actively growing vegetative trichomes of all algae under study were separately spread on solid BG_{11} media containing 2–10 % agar or inoculated in liquid BG_{11} media containing 0.1–1.0 mol/L NaCl, and kept in a culture chamber under normal culture conditions. The controls were maintained in liquid BG_{11} media. The survival of *O. acuminata* trichomes was determined by counting the percentage of living trichomes out of about 4000 trichomes. Formation of akinetes, heterocysts and dead cell count (if any) in *A. iyengarii*, *W. prolifica* and *N. lobatus* was estimated by counting their percentages relative to the total number of about 5000–6000 vegetative cells.

Viability of akinetes formed under water stress. The akinetes of *A. iyengarii*, *W. prolifica* and *N. obatus* formed on different agarized media and in NaCl-supplemented liquid media were harvested, washed with distilled water, and inoculated into liquid BG_{11} media under normal culture conditions. Akinetes harvested from liquid BG_{11} medium and similarly inoculated served as controls. The per cent germination of akinetes was determined on day 23 of inoculation in each case by counting about 3000 akinetes.

Table I. Per cent survival of dried vegetative trichomes of *O. acuminata*, *A. iyengarii*, *W. prolifica* and *N. lobatus* and dried akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* stored in desiccators over fused calcium chloride for different time periods at different light conditions and temperatures[a]

Storage time	Storage conditions				Storage time	Storage conditions			
	light[b]	dark				light[b]	dark		
		20 °C	12 °C	0 °C			20 °C	12 °C	0 °C
	O. acuminata, vegetative trichomes					*A. iyengarii*, vegetative trichomes			
1 h	100	100	100	8	1 h	100	100	100	0
	48	50	20	10		0	0	0	0
2 h	100	100	90	0		*A. iyengarii*, akinetes			
	30	31	17	0	1 mon	50	51	48½	14
3 h	100	100	87	—		53	50	48	13
	21	18	10½	—		*W. prolifica*, vegetative trichomes			
6 h	100	100	73	—	1 h	100	100	100	0
	20	15	10	—		3	2	1	0
½ d	100	100	70	—		*W. prolifica*, akinetes			
	15	11	9	—	1 mon	48	68	46	12
1 d	100	100	70	—		48	50	47	20
	12	15	8	—		*N. lobatus*, vegetative trichomes			
2 d	78	71	0	—	1 h	100	100	100	0
	0	0	0	—		0	0	0	0
						N. lobatus, akinetes			
					1 mon	47	49	41	10
						49	48	48½	19

[a]Measurement 15 d after inoculation in liquid BG_{11}. Controls were maintained in distilled water and kept under similar storage conditions. All values represent rounded means of three replicates. *First lines* — control, *second lines* — dried stage.
[b]Storage under 10-h illumination with fluorescent light of 1.4 klx at 20 °C.

Akinete germination under water stress. Freshly formed akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* in liquid BG_{11} media were transferred to 2–10 % agarized media and to 0.1–0.8 mol/L

Table II. Influence of agar BG_{11} media and NaCl-containing liquid BG_{11} media on per cent survival of vegetative trichomes of *O. acuminata* (L), and per cent akinete formation (A), heterocyst formation (H) and dead cell count (D) in *A. iyengarii*, *W. prolifica* and *N. lobatus*[a]

Days after inoculation	L, A, H, D %	Agar, %						NaCl, mol/L							
		0	2	4	6	8	10	0	0.10	0.15	0.20	0.25	0.50	0.80	1.0
							O. acuminata								
15	L	100	100	100	100	100	10	100	100	100	99	80	90	12	0
30	L	100	100	100	15	8	0	100	100	100	92	75½	60	10	–
45	L	100	100	51	20	10	–	–	–	–	–	–	–	–	–
60	L	100	100	30	18	8	–	100	100	100	90	70	42½	0	–
							A. iyengarii								
15	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	2	2	1	0	0	0	2	2	0	0	0	0	0	0
	D	0	0	0	15	42	100	0	0	15	20	100	100	100	100
30	A	2	1	0	0	0	–	1½	0	0	0	–	–	–	–
	H	2	2	1	0	0	–	2	2	0	0	–	–	–	–
	D	0	0	20	36	60	–	0	18	40	50	–	–	–	–
45	A	6	5	1	0	0	–	5	3	0	0	–	–	–	–
	H	2	2	1	0	0	–	2	2	0	0	–	–	–	–
	D	0	0	50	51	78	–	0	28	69	69	–	–	–	–
60	A	10	8	5	0	0	–	8	7	0	0	–	–	–	–
	H	2	2	1	0	0	–	2	2	0	0	–	–	–	–
	D	0	0	85	99	100	–	0	45	85	90	–	–	–	–
							W. prolifica								
15	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	1	1	1	1	0	0	1	1	1	1	1	½	0	0
	D	0	0	0	0	5	20	0	0	1	4	5	10	100	100
30	A	41	30	20	18	0	0	40	33	30	31	29	12	–	–
	H	1	1	1	1	0	0	1	1	1	1	1	½	–	–
	D	0	0	5	10	30	100	0	0	19	25	30	46	–	–

45	A	65	40	30	21	0	–	63	45	39½	38	36½	15	–	–
	H	1	1	1	1	0	–	1	1	1	1	1	½	–	–
	D	0	0	8	15	39	–	0	26	29½	39	46	60	–	–
60	A	99	59	38	25	0	–	99	49	47	44	39	19½	–	–
	H	1	1	1	1	0	–	1	1	1	1	1	½	–	–
	D	0	0	10	26	100	–	0	50	52	55	60	80	–	–
N. lobatus															
15	A	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	H	2	2	2	0	0	0	2	1½	1	1	1	1	0	0
	D	0	0	0	0	0	2	0	0	0	0	1	10	100	100
30	A	15	8	5	0	0	0	23	20	10	12	12	10	–	–
	H	2	2	2	½	0	0	2	2	1	1	1	1	–	–
	D	0	0	10	18	15	40	0	10	18	20	22	30	–	–
45	A	50	39	20	5	0	0	58	49	45	40	40	25	–	–
	H	3	3	2	1	0	0	2½	2	1	1	1	1	–	–
	D	0	5	17	20	60	100	0	19½	23	38	38	50	–	–
60	A	97	60	25	20	0	–	96½	70	61	58	50	29	–	–
	H	3	2½	2	1	0	–	2½	2	1	1	1	1	–	–
	D	0	8	20	30	100	–	1	28	38	41	49	70	–	–

[a]All values represent rounded means of three replicates.

NaCl-supplemented liquid media and placed in a culture chamber under normal culture conditions. The controls were maintained in liquid BG_{11} media. They were examined at intervals from the start of the experiment so as to determine the per cent germination of akinetes. The emergence of protuberances which subsequently developed into a trichome was taken as a criterion for germination.

RESULTS AND DISCUSSION

Viability of dried vegetative trichomes and akinetes. Almost all dried vegetative trichomes of *A. iyengarii*, *W. prolifica* and *N. lobatus* died within 1 h of storage. Those of *O. acuminata* retained viability to some extent for 1 d under similar storage conditions (Table I). The viability decreased sharply within 1 h of storage, then slowly up to 1 d and disappeared within 2 d of storage (Table I). Desiccated vegetative cells of *Anabæna cylindrica* could survive even up to 10 d of storage over silica gel (Yamamoto 1975). All the four blue-green algae were more sensitive to water stress than *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* (Agrawal and Singh 1999), probably due to their delicate trichomes which had either no sheath or a very diffluent sheath and a wall chemically and physically different from that of green algae.

The viability of dried vegetative trichomes of *O. acuminata* decreased more or less equally when stored at 20 °C either in the light or the dark but was lost rapidly with temperatures drop to 12 and 0 °C in the dark (Table I). The vegetative trichomes of *A. iyengarii*, *N. lobatus* and *W. prolifica* were more sensitive to frost than those of *O. acuminata* (Table I); this correlated with their sensitivity to desiccation which also involved osmotic stress and decreased availability of liquid water (Levitt 1958; Hawes *et al.* 1992).

Both dried and wet akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* were about equally viable when stored at 20 °C in the light or dark or at 12

and 0 °C in the dark but their germination ability decreased on storage and at 0 °C (Table I). Sili *et al.* (1994) observed that the viability of *Cyanospira* akinetes was not affected by desiccation.

Survival of vegetative O. acuminata trichomes, formation of akinetes and heterocysts of other algae under water stress. The per cent survival of *O. acuminata* trichomes strongly decreased in media containing 4–10 % agar and in 0.2–0.8 mol/L NaCl-supplemented media; all trichomes died in 10 % agar media within 30 d of inoculation and in 1.0 mol/L NaCl-supplemented liquid media within 15 d of inoculation (Table II). The breadth of *O. acuminata* trichomes increased from 4½ μm in control to 5¼ and 6¾ μm when they were grown in 0.5 and 0.8 mol/L NaCl-supplemented media. The trichomes developed a very thin sheath-like structure when grown in 0.8 mol/L NaCl-supplemented liquid media (Fig. 1B). The unicellular blue-green alga *Microcystis firma* resisted 0.9 mol/L NaCl, accumulating large amounts of polyol and showing cell enlargement. The salt-dependent increase of the cell diameter may serve as an indicator of both polyol accumulation and salt resistance (Erdmann and Schiewer 1984). In blue-green algae, the resistance to high electrolyte concentration is caused by the formation of protective impervious or electrically charged envelopes (Hof Fremy 1933). Increased envelope thickness was also observed in *Chroococcidiopsis* from desiccated cultures, probably contributing to prevention of water loss (Caiola *et al.* 1996).

Table III. Per cent germination of akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* formed in liquid BG_{11} medium, on agar media and in NaCl-supplemented media, in liquid BG_{11} medium on 23 d of inoculation[a]

Akinetes harvested from								
liquid medium	agar media, %			NaCl media, mol/L				
	2	4	6	0.10	0.15	0.20	0.25	0.50
A. iyengarii								
69	70	70	–	67	–	–	–	–
W. prolifica								
76	75	73	70	72	72	76	75	70
N. lobatus								
65	66	61	64	60	61	65	64	65

[a]Values represent rounded means of three replicates.

The akinete formation in *A. iyengarii* on 2–4 % agar media and in 0.1 mol/L NaCl-supplemented liquid media was delayed and decreased as compared to controls. Most vegetative cells died without any akinete formation on media containing 6 % or more agar and in liquid media containing more than 0.15 mol/L NaCl within 60 d of inoculation (Table II). Similarly, the akinete formation in *W. prolifica* and *N. lobatus* was also strongly decreased on 2–6 % agar media and in 0.1–0.5 mol/L NaCl-supplemented liquid media as compared to control. All vegetative cells again died without any akinete formation on media with 8 % or more of agar within 60 d and in liquid media with 0.8 mol/L NaCl or more within 15 d of inoculation (Table II); hence water stress decreased akinete formation and induced cell death in all three blue-green algae. In this respect *A. iyengarii* was more sensitive, while *W. prolifica* and *N. lobatus* were about equally resistant. Evans (1958) had shown that survival of desiccation by pond algae has no relation to the production of spores. Barbiero and Welch (1992) observed that many species of *Nostocales* overwinter as vegetative filaments rather than as akinetes. Spore-forming blue-green algae were observed to be generally absent from desert floras (Cameron and Blank 1966). Roelofs and Oglesby (1970) concluded from their field and laboratory observations that akinete formation was not a result of unfavorable conditions, as akinetes were seen during the exponential phase of growth. Water stress also decreased akinete formation in *Pithophora œdogonia* (Agrawal and Singh 1999).

The heterocyst formation in *A. iyengarii*, *W. prolifica* and *N. lobatus* was very low throughout the observation period in controls, on low-agar media and in low-NaCl liquid media. It was completely suppressed on high-agar media and in high-NaCl liquid media (Table II); hence water stress is unfavorable to heterocyst formation. Stewart (1965) observed that in field conditions, nitrogen fixation by *Calothrix scopulorum* was reduced in summer when the alga became desiccated and peeled off the rock.

Viability of akinetes formed under water stress. *A. iyengarii*, *W. prolifica* and *N. lobatus* akinetes formed under water stress were equally viable as those formed under normal conditions (Table III). This resembles *P. œdogonia* akinetes (Agrawal and Singh 1999).

Table IV. Per cent germination of akinetes of *A. iyengarii*, *W. prolifica* and *N. lobatus* on agar BG_{11} media and in NaCl-supplemented liquid BG_{11} media[a]

Days after inoculation	Agar, %						NaCl, mol/L	
	0	2	4	6	8	10	0	0.10
				A. iyengarii				
10	12	8	5	0	0	0	10	0
15	48	35	12	0	0	0	21	0
25	70	51	20	0	0	0	70	0
				W. prolifica				
10	19	15	8	8	0	0	19	0
15	23	19	12	20	0	0	25	0
25	82	65	51	25	0	0	80	0
				N. lobatus				
10	8	5	3	1	0	0	12	0
15	21	17	16	11	0	0	35	0
25	65	60	50	22	1	0	60	0

[a]Rounded means of three replicates.

Germination of akinetes under water stress. The per cent akinete germination in *A. iyengarii*, *W. prolifica* and *N. lobatus* decreased progressively on media containing 2–4, 2–6 and 2–8 % agar. At higher agar contents and at 0.1 mol/L NaCl or more the akinetes failed to germinate (Table IV); hence akinete germination in all three blue-green algae was more sensitive to physiological water stress than akinete formation (Table IV and II).

The authors thank the Head, *Department of Botany, University of Allahabad*, for providing laboratory facilities and the *University Grants Commission*, New Delhi, for financial assistance in the form of a research project.

REFERENCES

AGRAWAL S.C., SINGH V.: Viability of dried vegetative cells and the formation and germination of reproductive structures in *Pithophora œdogonia*, *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* under water stress. *Folia Microbiol.* **44**, 63–70 (1999).

BARBIERO R.P., WELCH E.B.: Contribution of benthic blue-green algae recruitment to lake populations and phosphorus translocation. *Freshwater Biol.* **27**, 249–260 (1992).

CAIOLA M.G., BILLI D., FRIEDMANN E.I.: Effect of desiccation on envelopes of the cyanobacterium *Chroococcidiopsis* sp. (*Chroococcales*). *Eur.J.Phycol.* **31**, 97–105 (1996).

CAMERON R.E., BLANK G.R.: *Desert algae: soil crusts and diaphanous substrata as algal habitats.* Jet Propulsion Laboratory Pasadena Tech. Rep. No. 32-971, pp. 1–45. Pasadena (USA) 1966.

ERDMANN N., SCHIEWER U.: Cell size changes as indicator of salt resistance of blue-green algae. *Arch.Hydrobiol.* (Suppl.) **67**, 431–439 (1984).

EVANS J.H.: The survival of freshwater algae during dry periods I. An investigation of the algae of five small ponds. *J.Ecol.* **46**, 149–168 (1958).

HAWES I., HOWARD-WILLIAMS C., VINCENT W.F.: Desiccation and recovery of antarctic cyanobacterial mats. *Polar Biol.* **12**, 587–594 (1992).

HOF T., FREMY P.: On *Myxophyceae* living in strong brines. *Rec.Trav.Bot.Neerland* **30**, 140–162 (1933).

LEVITT J.: Frost, drought and heat resistance, pp. 1–87 in *Protoplasmatologia-Handbuch der Protoplasma Forschung* (L.V. Heilbrunn, F. Weber, Eds), Vol. VIII. Springer, Vienna 1958.

ROELOFS T., OGLESBY R.T.: Ecological observations on the planktonic cyanophyte *Glœotrichia echinulata*. *Limnol.Oceanogr.* **15**, 224–229 (1970).

SCHERER S., ZHONG Z.P.: Desiccation independence of terrestrial *Nostoc commune* ecotypes (*Cyanobacteria*). *Microb.Ecol.* **22**, 271–283 (1991).

SILI C., ENA A., MATERASSI R., VINCENZINI: Germination of desiccated aged akinetes of alkaliphilic cyanobacteria. *Arch. Microbiol.* **162**, 20–25 (1994).

STANIER R.Y., KUNISAWA R., MANDEL M., COHEN B.: Purification and properties of a unicellular blue-green alga (order *Chroococcales*). *Bact.Rev.* **35**, 171–205 (1971).

STEWART W.D.P.: Nitrogen turnover in marine and brackish habitats. I. Nitrogen fixation. *Ann.Bot.* **20**, 229–239 (1965).

TOMASELLI L., GIOVANNETTI L.: Survival of diazotrophic cyanobacteria in soil. *World J.Microbiol.Biotechnol.* **9**, 113–116 (1993).

WHITTON B.A.: Survival and dormancy of blue-green algae, pp. 109–167 in *Survival and Dormancy of Microorganisms* (Y. Henis, Ed.). John Wiley & Sons, New York 1987.

YAMAMOTO Y.: Effect of desiccation on the germination of akinetes of *Anabœna cylindrica*. *Plant Cell Physiol.* **16**, 749–752 (1975).