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Survival and reproduction of some aquatic green algae facing various stress conditions

S. C. Agrawal* and Suman Gupta
Department of Botany, University of Allahabad, Allahabad- 211002, India. *Corresponding author. E-mail: 20.satish@gmail.com

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Abstract

Physical or physiological water stress (imparted by growing algae on agarized solid medium or in salinized liquid medium, respectively), absence of all nutrients in liquid medium(glass double distilled water), darkness, and low light intensity (of 2 and 10 µmol m^{-2} s^{-1}), heat shock (of ≥ 36°C for 5- 15 min), or UV exposure (of 0.384- 5.76 kJm^{-2}), all, decreased, differently, the percentage vegetative survival of aquatic green algae *Scenedesmus quadricauda* (Turp.) Breb., *Cosmarium granatum* Brebisson, *Hormidium flaccidum* (Kützing) Braun, *Oedogonium* sp. and *Spirogyra* sp.; and led *Sc. quadricauda* to shed all spines rapid, *H. flaccidum* filaments to fragment less or not at all, or *Oedogonium* sp. filaments cells to differentiate into oogonium- like cells unaltered or not at all. No stress conditions induced the formation of any perennating or reproductive cell in any alga studied.

Introduction

In nature, the survival and reproduction of aquatic green algae are possibly affected negatively when they are exposed to physical or physiological water stress, heat or UV when water bodies get dried in summer due to water evaporation. Water stress inhibited the vegetative survival and reproduction in aquatic green algae *Pithophora oedogonia, Cladophora glomerata, Rhizoclonium hieroglyphicum, Chlorella vulgaris* and *Hydrodictyon reticulatum* and did not induce conjugation in *Spirogyra* sp. (Agrawal and Singh 1999 a, 2001, 2002; Agrawal and Pal 2003; Gupta and Agrawal 2004). UV light produced cells shrinkage and decreases the reproduction in *Oedogonium cardiacum* (Parker and Horsley 1972), *Stigeoclonium pascheri* (Sarma and Agrawal 1980) and *Chlamydomonas reinhardtii* (Moharikar *et al.* 2006). The present study reports the vegetative survival, morphological features and reproduction in aquatic green algae *Scenedesmus quadricauda* (Turp.) Breb., *Cosmarium granatum* Brebisson, *Hormidium flaccidum* (Kützing) Braun, *Oedogonium* sp., and *Spirogyra* sp. in relation to physical or physiological water stress, lack of all nutrients in liquid medium, darkness and low light intensity, heat shock or UV exposure.

Material and Methods

Sc.quadricauda coenobia, *C. granatum* mucilage masses of vegetative cells, *H. flaccidum* filaments, *Oedogonium* sp. filaments, and *Spirogyra* sp. filaments were collected while growing inside different shallow freshwater bodies in Allahabad, India. Their clonal cultures were maintained in liquid Bold's basal medium, BBM (Nichols and Bold 1965; pH adjusted prior to autoclaving to 7.5) at 22 ± 1°C and light intensity of 40 µmol m^{-2} s^{-1} from daylight fluorescent tubes for 16 h a day in a culture chamber. Per cent vegetative survival in different algae was determined by counting the number of live coenobia (in *Sc. quadricauda*), live vegetative cells (in *C. granatum*), or live vegetative cells of filaments (in rest of all other algae) relative to dead coenobia or dead vegetative cells (looking hyaline empty distorted, deformed or disintegrated out of *ca.* 500- 7000 coenobia/ vegetative cells/ vegetative cells of filaments from each of 3 replicates.

Morphological and reproductive features of different algae in control: *Sc. quadricauda* 4- cells coenobia had 4 spines per coenobium in 0 to 15 day old cultures, but they had mostly 2, 1, and 0 spines per coenobium in 20, 25, and 30 day old cultures,

respectively (the alga shed off spines in old age cultures). *C. granatum* vegetative cells did not conjugate in any age old cultures. *H. flaccidum* population had 3, 4, 10, 15, 8, 3, and 0 % fragments (up to 5 cells long) and rest long filaments in 3, 5, 10, 20, 25, 30, and 38 day old cultures, respectively (fragmentation of filaments occurred maximally during growth period and was decreased in old age cultures because of slow or no growth). *Oedogonium* sp. filaments had 1 % of all cells, oogonium- like (42.9 µm broad than the 19.8 µm broad vegetative cells) in 20 to 40 day- old cultures, but had no antheridium- like cell in any- age old cultures. *Spirogyra* sp. filaments did not reproduce in any- age old cultures.

***Algal inoculants*:** Three- day old control cultures of *Sc. quadricauda*, *C. granatum*, *H. flaccidum*, and *Oedogonium* sp., and one- day old control culture of *Spirogyra* sp. (surviving almost cent per cent) were used as a source of inoculate for all experiments. Inoculum's of *Sc. quadricauda* had 4 spines per coenobium, that of *C. granatum* had all cells vegetative, and of *H. flaccidum* had all filaments long and no fragment (fragments were washed off in running water and only long filaments retained), of *Oedogonium* sp. filaments had all cells vegetative and no oogonium- like cells; and that of *Spirogyra* sp. filaments had all cells vegetative.

***Physical and physiological water stress*:** Different algal inoculate were separately spread on solid Bold' basal media containing 1- 8 % agar (highly agarized media impart physical water stress) or inoculated into liquid media containing 0.1- 0.5 M NaCl (salinized liquid media impart physiological water stress) and kept in the culture chamber under normal culture conditions. Vegetative survival, morphological features, and reproduction if any, of different algae were investigated periodically.

***Absence of all nutrients*:** To omit all nutrients, glass double- distilled water was used as a culture medium. Its pH was adjusted to 7.5 prior to autoclaving. Acid-washed Borosil glassware properly rinsed with double distilled water were used. Algal inoculants, washed carefully with sterile glass double- distilled water were separately inoculated into sterilized glass double-distilled water and kept in the culture chamber.

***Darkness and low light intensity*:** Different algal inoculate suspended in liquid media were exposed to (i) darkness by wrapping the inoculated culture tubes with black paper and keeping them in the dark in the culture chamber, and (ii) at low light intensity of 2 and 10 µmol m^{-2} s^{-1} by adjusting the distance of the inoculated culture tubes from the light source in the culture chamber. Control cultures were kept at light intensity of 40 µmol m^{-2} s^{-1} in the culture chamber. The light intensity was measured with a Lutron lux meter and converted into µmol m^{-2} s^{-1}. Vegetative survival, morphological features, and reproduction if any, of different algae were assessed periodically.

Heat shock: Different algal inoculants were given heat treatment (of ≥ 36˚C for 5- 15 min) submerged in sterile distilled water and kept in hot water bath. After heat treatment, algal materials were inoculated in sterile liquid culture medium and transferred to culture chamber and observed for vegetative survival, morphological features and reproduction till they survive. The controls had no heat treatment but were treated similarly (were submerged in distilled water for 15 min, the maximum time of any heat treatment) and then inoculated into sterile liquid culture medium and kept in the culture chamber.

***UV exposure*:** All algal inoculants, separately, placed in 10 ml sterile media, spread in open Petri dishes (diameter 90 mm) were exposed to UV light from a *Philips* germicidal lamp (main output at 254 nm and a fluence rate of 3.2 W m^{-2}). The energy fluence of UV light which was obtained by increasing the time of exposure from 2 to 30 min ranged from 0.382 to 5.76 k J m^{-2}. After irradiation, the algal materials were transferred directly to culture chamber and assessed periodically for vegetative survival, morphological features and reproduction. Materials not UV-irradiated served as controls.

Results and Discussion

***Physical and physiological water stress*:** Physical or physiological water stress produced early death of all aquatic green algae assessed (Table 1) without induction of any resistant or perennating cell. No algal cell revived (appeared alive) if fresh liquid medium was pour over dead algae on the dried agar surface, or the dead algae in any of the salinized- liquid media were removed, washed with distilled water, and transferred to fresh liquid media, and allowed to grow for a month or more in the culture chamber. The osmotic potentials of green algal cells were found to be equivalent to 0.11, 0.17 M NaCl (Lothring 1941-42). Among the present aquatic green algae, *Spirogyra*

sp. was the most sensitive to both physical and physiological water stress (Table 1). All present aquatic green algae were much more sensitive to both physical and physiological water stress than any of terrestrial blue- green algae *Scytonema millei*, *Phormidium bohneri*, *Lyngbya mesotricha* and *Microcoleus chthonoplastes* (Gupta and Agrawal 2006).

Table1. Percentage vegetative survival of different green algae as influenced by physical (agarized solid BBM) and physiological water stress (NaCl- containing liquid BBM) [a].

Alga	Time (d)	Control [b]	Agar (%)					NaCl (M)					
			1	2	4	6	8	0.1	0.15	0.2	0.3	0.4	0.5
Sc. quadricauda	3	100	98	98	78	59	30	98	93	79	63	-	-
	5	100	86	83	59	38	9	80	76	60	33	-	-
	7	98	69	66	38	12	0	69	62	43	10	-	-
	10	87	44	42	19	0	-	50	48	20	1	-	-
	20	67	7	8	-	-	-	18	2	-	-	-	-
	25	50	-	-	-	-	-	0	-	-	-	-	-
C. granatum	3	98	88	78	64	48	23	96	88	71	58	36	10
	5	82	70	65	41	27	12	80	70	60	31	7	-
	7	66	42	39	24	10	0	69	59	39	7	-	-
	10	47	23	22	10	0.5	-	50	32	18	-	-	-
	15	29	11	12	-	-	-	29	9	0	-	-	-
	20	8	0	-	-	-	-	5	-	-	-	-	-
H. flaccidum	3	100	98	98	86	69	40	98	98	71	63	62	50
	5	100	90	89	67	42	15	93	90	59	50	47	26
	7	97	79	79	45	18	2	89	84	44	40	20	3
	10	90	63	62	30	9	-	73	70	33	23	4	-
	20	70	27	22	0.5	-	-	52	39	-	-	-	-
	30	40	0.5	0	-	-	-	19	-	-	-	-	-
Oedogonium sp.	3	100	95	89	69	49	29	100	100	100	87	59	38
	5	100	89	70	41	25	13	100	100	98	70	37	23
	10	100	70	36	8	0	-	96	90	88	38	1	0
	20	99	29	2.5	-	-	-	89	78	68	0	-	-
	40	67	-	-	-	-	-	51	24	1	-	-	-
Spirogyra sp.	1	96	58	38	15	2	0	64	48	29	10	0	-
	3	64	32	11	0	-	-	32	20	-	-	-	-
	5	39	12	0	-	-	-	10	7	-	-	-	-
	7	9	0	-	-	-	-	-	-	-	-	-	-

[a] About 5000- 70000 coenobia in *Sc. quadricauda*, vegetative cells in *C. granatum*, and vegetative cells of filaments in rest of all other algae were assessed for each triplicate; data represent rounded means; for details *see Material and Methods*; Dash indicate that the alga was already dead.

[b] Algae grown submerged in BBM at 40 µmol m^{-2} s^{-1} light intensity for 16 h a day at 22 ± 1 ˚C in the culture chamber.

Physical and physiological water stress not only reduced the vegetative survival in all aquatic green algae assessed (Table 1), but also led *Sc. quadricauda* coenobia to shed all spines rapid, *H. flaccidum* filaments to fragment less or not at all, and *Oedogonium* sp. filaments cells to differentiate into oogonium- like cells either unaffected or not at all. *Sc. quadricauda* coenobium shed- off all spines on 4 % agarized solid medium and in 0.1- 0.3 M NaCl-containing liquid media within 5 day of inoculation; and had only 3, 1 and 0 spines on 2 % agarized solid medium on 5, 10, and 20 day of inoculation, respectively. *H. flaccidum* filaments population had no fragment (because of suppressed growth) on 6 % agarized solid medium and in 0.4 M NaCl- containing liquid medium till they survive, and had only 1 % of fragments on 4 % agarized solid medium (on 10 day of inoculation), and in 0.2 and 0.3 M NaCl- containing liquid medium (on 5 day of inoculation). *Oedogonium* sp. filaments did not differentiate into any oogonium-like cell on 2- 6 % agarized solid medium and in 0.3 or 0.4 M NaCl- containing liquid medium till they survive, but they had 1 % of all cells differentiated into oogonium- like cells in 0.1 or 0.2 M NaCl-containing liquid medium on 20 day after inoculation. Any level of physical and physiological water stress did not induce the formation of any antheridial cell in *Oedogonium* sp., and conjugation in *C. granatum* vegetative cells or in *Spirogyra* sp. filaments.

Water stress suppressed the formation of akinetes in *Pithophora oedogonia*, *Anabaena iyengarii*, *Westiellopsis prolifica* and *Nostochopsis lobatus*; inhibited cell division in *Chroococcus minor*, *Gloeocapsa aeruginosa* and *Aphanothece nidulans*; inhibited formation of hormogonia in *Lyngbya martensiana*; false branch and heterocyst in *Scytonema hofmanni*; daughter net in *Hydrodictyon reticulatum*; fragmentation in *Hormidium fluitans*; and autospores release in *Chlorella vulgaris* (Mahmoud *et al*. 1992; Agrawal and Singh 1999 a, b, 2001, 2002; Agrawal and Pal 2003).

***Absence of all nutrients*:** All aquatic green algae survived less and die early (Table 2) without induction of any reproductive or perennating cell in absence of all nutrients of the culture medium (i.e. in glass double- distilled water). *Sc. quadricauda* coenobia shed off all spines early (within 5 day after inoculation), *H. flaccidum* filaments had only 2 % of fragments (as compared to 10 % in BBM) on 10 day of inoculation, and *Oedogonium* sp. filaments cells died without formation of any oogonium- like cells in absence of all nutrients of the culture medium. Lack of all nutrients decreased viability and germination of akinetes in *A. iyengarii*, *W. prolifica*, *N. lobatus* and *P. oedogonia*, and reduced germination of zoospores in *C. glomerata* and *R. hieroglyphicum* (Agrawal and Misra 2002).

Table 2. Percentage vegetative survival of different aquatic green algae in absence of all nutrients in liquid medium (glass double distilled water, DW) and in presence of darkness or low light intensity (of 2 and 10 µmol m^{-2} s^{-1}) [a].

Alga	Time (d)	Control [b]	DW	Light intensity (µmol m^{-2} s^{-1})		
				0	2	10
Sc. quadricauda	3	100	67	39	46	57
	5	100	30	14	23	42
	7	98	2	0	10	20
	10	87	-	-	-	1
C. granatum	3	98	60	42	59	66
	5	82	38	21	38	49
	7	66	19	2	16	26
	10	47	2	0	1	10
H. flaccidum	3	100	89	20	58	68
	5	100	70	0	34	40
	7	97	58	-	3	13
	10	90	37	-	-	0.5

Oedogonium sp.	3	100	94	59	69	76
	5	100	81	40	42	51
	10	100	48	1	2	10
	20	99	2	-	-	-
Spirogyra sp.	1	96	15	40	52	56
	3	64	-	0	8	6

[a, b] Same as that in Table 1.

Darkness and low light intensity: All aquatic green algae survived less and died early in response to darkness and at low light intensity of 2 and 10 µmol m^{-2} s^{-1} (than at 40 µmol m^{-2} s^{-1} of light intensity in control culture, Table 2). Darkness and low light intensity of 2 and 10 µmol m^{-2} s^{-1} impart shedding of all spines in *Sc. quadricauda* early (within 5 days in darkness and at low light intensity of 2 µmol m^{-2} s^{-1}, and within 7 days at low light intensity of 10 µmol m^{-2} s^{-1}). Darkness and low light intensity (of 2 and 10 µmol m^{-2} s^{-1}) induced no conjugation in *C. granatum* cells, no fragmentation in *H. flaccidum* filaments, no oogonium- like cell formation in *Oedogonium* sp. filaments, and no perennating or reproductive cell formation in any algae studied.

Chlorella vulgaris cells required the light intensity of more than 10 µmol m^{-2} s^{-1} for the formation of autospore mother cells (Agrawal and Singh 2001). Low light had an inhibitory effect on the cell division in *C. minor*, *G. aeruginosa*, and *A. nidulans*; heterocyst and false branch formation in *Scytonema hofmanni*: and on the daughter net formation in *Hydrodictyon reticulatum* (Agrawal and Pal 2003).

***Heat shock* :** All present aquatic green algae survived less and died early than in controls when exposed to heat shock of 36 ˚C for 5- 15 min, 45 ˚C for 5- 10 min, and 50 ˚C for 5 min (Table 3). Heat shock of any extent and duration used in the present study led *Sc. quadricauda* coenobia to shed all spines following heat treatment, no conjugation induction in heat- survived *C. granatum* vegetative cells, very less or no fragmentation in *H. flaccidum* filaments and no oogonium- like cell formation in *Oedogonium* sp. filaments cells. *H. flaccidum* filaments population had only 4 and 1 % of fragments on 7 day of inoculation following heat treatment of 36 ˚C for 5 and 10 min, respectively (in control culture without any heat treatment, it was 8 % of fragments on 7 day of inoculation). Heat shock of 45 ˚C for 10 min led all filaments of *H. flaccidum* dead without any fragmentation. Heat shock of 40 ˚C (for 15- 60 min) inhibited cell division in *C. minor*, *G. aeruginosa* and *A. nidulans*; heterocyst formation in *Scytonema hofmanni*; and daughter net formation in *H. reticulatum* (Agrawal and Pal 2003). *Rhizoclonium riparium* vegetative filaments disintegrated within a week following exposure to 40 ˚C for 24 h (Hall and Walmsley 1991). Exposure of *C. glomerata* vegetative filaments to 28.5 ˚C for 12 h stops zoosporangium-primordium formation (Gupta and Agrawal 2007).

***UV exposure*:** UV light (of 0.384 to 5.76 kJm^{-2} dose) led all present aquatic green algae to survive less and die early (Table 4), *Sc. quadricauda* coenobia to shed all spines rapid (no spine was observed following UV irradiation of any dose), no induction of conjugation in *C. granatum* vegetative cells, less or no fragmentation in *H. flaccidum* filaments popuation (maximum only 5 and 2 % of fragments occurred following 0.384 and 0.96 kJm^{-2} of UV dose, respectively; but it was maximum 15 % in the control culture. No fragmentation occurred following ≥ 1.92 kJm^{-2} of UV dose), and no differentiation into oogonium- like cells in *Oedogonium* sp. filaments cells. UV light decreased the chlorophyll levels in *Chlorella pyrenoidosa* (Chen *et al.* 2003).

Table 3. Death day of different aquatic green algae in control culture as well as following heat treatment for different durations [a]

Alga	Control	Heat treatment					
		36˚C			45 ˚C		50 ˚C
		5	10	15 min	5	10 min	5 min
Sc. quadricauda	41	16	15	9	9	4	≤ 1
C. granatum	25	20	17	11	14	8	4
H. flaccidum	41	27	24	16	25	12	3
Oedogonium sp.	63	36	30	23	11	8	6
Spirogyra sp.	9	5	2	1	2	≤ 1	≤ 1

[a] Control had no heat treatment but had materials submerged in sterile distilled water for 15 min (the maximum time for any heat treatment) in the laboratory and then inoculated in sterile liquid BBM and kept in the culture chamber. During heat treatment the materials were submerged in sterile distilled water and thereafter were transferred to sterile BBM and kept in the culture chamber. Death day represent the time when all the cells of the algal population were dead.

Table 4. Survival period (in day) of different algae following UV exposure of 0.384- 5.76 kJm^{-2} dose [a]

Alga	Control	UV exposure (kJm^{-2})						
		0.384	0.96	1.92	2.88	3.84	4.80	5.76
Sc. quadricauda	40	36	35	16	12	7	5	2½
C. granatum	24	18	17	8½	6	3	≤ 1	-
H. flaccidum	40	28	20	10	5	≤ 1	-	-
Oedogonium sp.	62	23	14	11	10	7	3	2
Spirogyra sp.	8	7	5	≤ 1	-	-	-	-

[a] All algae maintained in culture chamber following UV exposure. Control had no UV treatment. Dash indicates that the material was already dead.

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