Assessing the vitality of Himalayan lichens by measuring their photosynthetic performances using chlorophyll fluorescence technique

Sanjeeva Nayaka[1,*], Sanjay Ranjan[2], Pooja Saxena[2], U. V. Pathre[2], D. K. Upreti[1] and Ruchi Singh[2]

[1]Lichenology Laboratory, and [2]Plant Physiology Laboratory, National Botanical Research Institute (Council of Scientific and Industrial Research), Rana Pratap Marg, Lucknow 226 001, India

Photosynthetic performances of 82 lichens occurring in Western Himalayas were determined using chlorophyll fluorescence. F_v/F_m ranged from 0.023 to 0.655, with terricolous *Cladonia subconistea* at alpine region having maximum value. Photosynthetic performances of alpine lichens were found to be better than those of temperate due to the influence of favourable climate, wet soil and rock in the region. As the study was carried out during early summer, most of the lichens started experiencing stress which is evident by their F_v/F_m values. As many as 10 chlorolichens (with green alga as photobiont) growing in temperate region are severely stressed and have values <0.1. The stress components in the study area are mostly water availability and high intensity light. The cyanolichens (with blue green alga as photobiont) have relatively lower F_v/F_m ranging from 0.075 to 0.315. On the basis of their F_v/F_m values, the lichens in the present study are classified into three categories: normal, moderate and severely stressed with values ranging from 0.5 to 0.76, 0.3 to 0.49 and 0.01 to 0.29 respectively. The possible influence of crowded apothecia, growth form, substratum and association with moss towards the photosynthetic performance of lichens and their community are discussed.

Keywords: Chlorophyll fluorescence, ecophysiology, F_v/F_m, lichen, Himalayas.

PHOTOSYNTHESIS is the core physiological function of an autotrophic organism and its functional state has been considered as an ideal tool to monitor the health and vitality of plants[1]. Between gas exchange and chlorophyll fluorescence methods for measuring the photosynthetic performance in a plant, the latter technique has become more popular in the recent years. Introduction of highly user-friendly portable fluorometers further widened the scope of photosynthesis research, especially in *in situ* conditions. The technique was also found to be useful for studying samples such as lichens and bryophytes, whose structure otherwise makes them difficult to study with conventional gas exchange systems[2]. The chlorophyll fluorescence technique provides a large amount of data with a minimum of expertise and time, and without injury to the plants. The technique most frequently utilizes the parameter F_v/F_m as a reliable indicator of the maximum photochemical quantum efficiency of photosystem II or photosynthetic performance of organism under investigation[3,4]. It is the only parameter that is not temperature sensitive and measured in dark-adapted samples. F_v/F_m is calculated from F_0, the fluorescence when the reaction centre of PSII is full open and F_m, the maximum fluorescence when all the reaction centres are closed following a flash of saturation light. F_v (i.e. $F_m - F_0$) is the maximum variable fluorescence in the state when all non-photochemical processes are at a minimum[5].

Chlorophyll fluorescence technique has been effectively utilized as an indicator of photosynthetic performance in lichens too. It has proved particularly useful tool for vitality screening of lichen, not only in the laboratory but also in the field[6]. The photosynthetic response has been reported in lichens exposed to extreme temperature, light, water availability[7], air pollution, heavy metal concentration and UV-B[8–12]. Jensen[6], and Jensen and Kricke[13] described in detail the procedure for measuring chlorophyll fluorescence in lichens along with the advantages and limitations of each parameter.

India, being a hotspot for lichen diversity, made considerable progress in taxonomic and floristics studies and represents about 2450 species with high degree of endemism[14,15]. The Western Ghats in South India and the Eastern Himalayas with mostly tropical climate possess maximum diversity of lichens (949 and 759 spp. respectively), whereas the Western Himalayas (550 spp.) with temperate climate has luxuriant growth of lichen[16,17]. The availability of diverse climate, vegetation, topography and substratum in particular, makes India one of the lichen-rich countries with ecologically and physiologically interesting mycota. However, the ecophysiological aspects of Indian lichens are hitherto not studied and hence their physiological responses to various ecological

*For correspondence. (e-mail: sanjeeva_n@yahoo.com)

conditions are not known. Lichens are sensitive to changes in the microclimatic conditions and conclusive ecophysiological information is very much necessary to understand the reasons for their varied diversity, abundance, distribution and response to climate change. Ecophysiological studies can also yield a list of lichens that are ecologically wide niched and physiologically better performers, maybe in terms of growth and response to stress. Such lichens can be utilized for bioprospecting, especially gene mining for drought tolerance, as availability of water is the major concern of modern days. Keeping these facts in view, we initiated ecophysiological studies on Indian lichens to observe general trend and to generate baseline data. To begin with, the Western Himalayan region was selected owing to its easy accessibility and interesting lichen mycota. In the present communication, vitality of Himalayan lichens is assessed with fluorescence parameter F_v/F_m.

Materials and methods

Study area

The study area is situated in the midst of Western Himalayas, in a remote village called Munsiyari of Pithoragarh District, Uttarakhand (Figure 1). Two sites, one in temperate (Bujani, 30°03.675′N, 080°12.961′E, alt. 2800 m) and another in alpine region (Khaliya Top, 30°03.806′N, 080°12.182′E, alt. 3500 m) along the trekking route Balanti Band to Khaliya Top were explored for lichen collection and chlorophyll fluorescence study. The forest in the temperate region is dominated by *Quercus* and *Rhododendron* trees with few exposed rocks, whereas the alpine region included grassland with large boulders. The study was carried out during the early summer (April 2008) when a large amount of winter snow and glaciers are in the process of melting in the alpine region leaving the soil and rock wet. The diurnal temperature, light and relative humidity at temperate region during lichen collection varied between 10°C and 30°C, 08 and 2800 μmol m^{-2} s^{-1} and 20 and 40% respectively, whereas in the case of alpine region they varied from 5°C to 20°C, 10 to 3000 μmol m^{-2} s^{-1} and 20 to 30%.

Lichen sampling, pretreatment and identification

A total of 82 lichen samples were collected from both temperate and alpine regions. The lichens were growing on bark, rock and soil in the temperate region, whereas the alpine regions had only rock and soil as major substratum (Table 1). Though there were several more lichens in the area for the present study, only the samples those are morphologically distinct and devoid of mixtures were collected. The crustose lichens were growing directly on their substratum, whereas a few saxicolous foliose lichens were found growing over moss on the rocks. Otherwise in many cases, especially with fruticose lichens, moss was found to be the common associate. Lichen samples were collected along with their substratum using chisel causing minimum damage to the thallus. The collected lichen samples were brought to the laboratory by wrapping them with moist cloth, and were kept in growth chamber under temperature, light, relative humidity of 12°C, 20–30 μmol m^{-2} s^{-1} (12 h light and dark cycle) and 70% respectively. Distilled water was sprayed over the specimens once a day. The specimens were identified by studying their external morphology, internal morphology and chemistry following Awasthi[18,19] and Orange *et al.*[20]. Care was taken not to expose the samples to the external environment for longer duration during microscopic

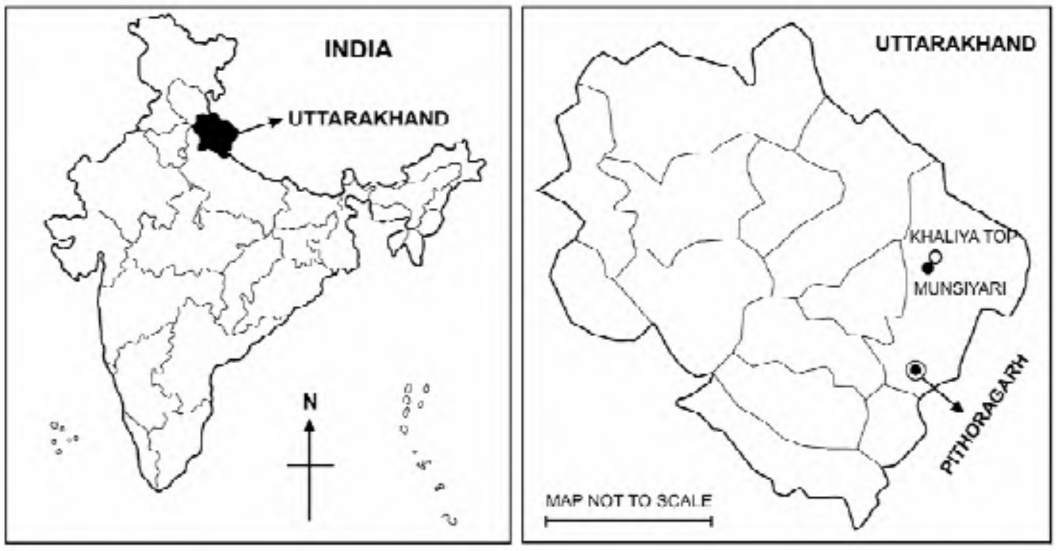

Figure 1. Map of India and Uttarakhand showing the study area Balanti Band–Khaliya Top.

Table 1. Numerical presentation of lichen flora of Balanti Band–Kaliya Top (Munsiyari)

	Both the regions		Temperate		Alpine	
Community	Specimens	Species	Specimens	Species	Specimens	Species
All	82	64	61	48	21	17
Chlorolichen	68	57	49	42	20	16
Cyanolichen	13	7	12	6	1	1
Crustose	28	23	19	16	9	8
Foliose	36	27	31	21	5	5
Fruticose	18	14	11	9	7	4
Bark	38	34	38	35	0	0
Rock	36	30	22	17	14	13
Soil	7	5	1	1	7	4

study while identification and, in many cases, a small part or duplicates of the samples were used.

Fluorescence measurements

It was not possible to take fluorescence measurements in the field as approach to the study area involved tedious trekking with steep climbing. Fluorescence measurements were taken after maintaining the lichen samples for three days in the above mentioned conditions. The lichen samples were moistened with a spray of distilled water and covered with black cotton cloth for 30 min (dark adaptation) and chlorophyll fluorescence was measured using PAM-2000 (Walz, Germany) with saturation pulse of 4000 photons μmol m^{-2} s^{-1} for 0.8 s. A total of 10 readings each were taken randomly on thallus for all the samples. Only F_v/F_m values were taken in the study, whereas F_0 and F_m were excluded, as they were sensitive to temperature and instrumental settings.

PAM-2000 is an excellent equipment for measuring various parameters of photosynthesis. Apart from being portable, it can take the measurements of a small thallus <1 cm in size. It can also measure chlorophyll fluorescence of leprose lichens with scattered granules as in the case of *Chrysothrix chlorina* under study. Schroeter *et al.*[21] are probably the pioneers to use PAM-2000 for measuring chlorophyll fluorescence in lichens and explained advantages of the equipment and importance of moisture in lichen thallus during the measurements. They measured fluorescence yield of crustose lichen *Buellia frigida* in Antarctica and found inhomogeneous distribution of water in the thallus during a drying cycle.

Statistical analysis

Average of F_v/F_m was calculated for all the lichens (Table 2) along with the standard deviation. For data analysis and discussion, two groups of communities were defined: (i) based on their growth forms (crustose, foliose, fruticose communities) and (ii) based on the substrates (corticolous, terricolous and saxicolous). Means of F_v/F_m of communities were compared using Student's *t*-test and ANOVA and P value <0.05 was considered as significantly different. The analyses were carried out for all the communities for pooled data (for both alpine and temperate) and independently for temperate and alpine region. All the statistical analyses were carried out using software SAS 9.1.3 (SAS Institute Inc., Canj, NC, USA).

Results

Lichen flora

The area between Balanti Band and Khaliya Top consists of interesting lichen flora and is estimated to have more than 100 species (from unpublished data). However, due to our selective sampling, identification of 82 lichen samples resulted in 64 species (Tables 1 and 2). Though there is representation of more number of lichen samples in the temperate region, the proportion of species composition is almost similar in alpine regions (temperate – 79%, alpine – 81%). Out of 13 samples of cyanolichens, 12 (six spp.) are present in the temperate region and only *Coccocarpia erythroxyli* belongs to alpine region. The temperate region represented more number of foliose lichens (31) followed by crustose (17), whereas in the alpine region more representation of crustose lichens was noticed (9), followed by fruticose lichens (7). Similarly, the temperate region is represented by more number of corticolous lichens (38), then by saxicolous (17) and *Cladonia scabriuscula* is the only terricolous lichen. In the case of alpine region, rock and soil being major substrata, the saxicolous lichens are represented more (14 spp.), but the terricolous lichens are also conspicuous (7). The genus *Cladonia* is more dominant in the present collection, distributed equally in both the regions and found growing mostly on soil. Parmeliaceae and Physciaceae are the most dominant families in the studied area.

Table 2. Lichens studied are listed in the decreasing order of F_v/F_m, their growth form, substratum, photobiont and distribution (CR = Crustose, FO = Foliose, FR = Fruticose, CHL = Chlorolichen alga, CYA = Cyanolichen)

Lichen taxa	Growth form	Substratum	Photobiont	Distribution	F_v/F_m
Cladonia subconistea Asahina	FR	Soil	CHL	Alpine	0.655 ± 0.024
Cladonia subconistea Asahina	FR	Soil	CHL	Alpine	0.652 ± 0.015
Cladonia kurokawae Ahti and S. Stenroos	FR	Soil	CHL	Alpine	0.630 ± 0.035
Cladonia subconistea Asahina	FR	Soil	CHL	Alpine	0.626 ± 0.019
Cladonia sinensis S. Stenroos and J.B. Chen	FR	Rock	CHL	Temperate	0.623 ± 0.041
Porpidia hydrophila (Fr.) Hertel and A.J. Schwab	CR	Rock	CHL	Alpine	0.616 ± 0.028
Cladonia ochrochlora Flörke	FR	Bark	CHL	Temperate	0.596 ± 0.053
Phaeophyscia hispidula var. *exornatula* (Zahlbr.) Moberg	FO	Bark	CHL	Temperate	0.587 ± 0.057
Buellia indica S.R. Singh and D.D. Awasthi	CR	Rock	CHL	Temperate	0.552 ± 0.021
Cladonia scabriuscula (Delise) Leight.	FR	Soil	CHL	Temperate	0.536 ± 0.045
Hypotrachyna awasthii Hale and Patw.	FO	Bark	CHL	Temperate	0.530 ± 0.066
Dermatocarpon miniatum (L.) W. Mann	FO	Rock	CHL	Temperate	0.509 ± 0.128
Stereocaulon foliolosum Nyl.	FR	Rock	CHL	Alpine	0.508 ± 0.052
Pertusaria amara (Ach.) Nyl.	CR	Bark	CHL	Temperate	0.502 ± 0.079
Usnea nipparensis Asahina	FR	Bark	CHL	Temperate	0.498 ± 0.042
Cladonia crispata var. *cetrariiformis* (Delise) Vain.	FR	Soil	CHL	Alpine	0.482 ± 0.068
Cladonia scabriuscula (Delise) Leight.	FR	Rock	CHL	Temperate	0.480 ± 0.099
Cladonia subconistea Asahina	FR	Soil	CHL	Alpine	0.473 ± 0.038
Cladonia ceratophyllina (Nyl.) Vain.	FR	Rock	CHL	Temperate	0.468 ± 0.077
Cladonia cartilaginea Müll. Arg.	FR	Bark	CHL	Temperate	0.451 ± 0.169
Ochrolechia yasuda var. *corallina* Poelt	CR	Bark	CHL	Temperate	0.430 ± 0.047
Rinodina mackenziei Räsänen	CR	Bark	CHL	Temperate	0.428 ± 0.154
Acarospora saxicola Fink ex J. Hedrick	CR	Rock	CHL	Temperate	0.422 ± 0.071
Tephromela khatiensis (Räsänen) Lumbsch	CR	Rock	CHL	Alpine	0.419 ± 0.073
Verrucaria acrotella Ach.	CR	Rock	CHL	Temperate	0.413 ± 0.119
Porpidia macrocarpa (DC.) Hertel and A.J. Schwab	CR	Rock	CHL	Temperate	0.412 ± 0.159
Parmelinella wallichiana (Taylor) Elix and Hale	FO	Bark	CHL	Temperate	0.411 ± 0.161
Rhizocarpon geographicum (L.) DC.	CR	Rock	CHL	Alpine	0.403 ± 0.095
Lecanora cfr. *streimannii* Lumbsch	CR	Rock	CHL	Alpine	0.402 ± 0.123
Verrucaria acrotella Ach.	CR	Rock	CHL	Temperate	0.397 ± 0.052
Aspicilia almorensis Räsänen	CR	Rock	CHL	Alpine	0.395 ± 0.098
Pyxine sorediata (Ach.) Mont.	FO	Bark	CHL	Temperate	0.377 ± 0.057
Melanelia stygia (L.) Essl.	FO	Rock	CHL	Alpine	0.373 ± 0.100
Graphis longiramea Müll. Arg.	CR	Bark	CHL	Temperate	0.368 ± 0.169
Toninia sp.	CR	Rock	CHL	Alpine	0.365 ± 0.040
Ioplaca pindarensis (Räsänen) Poelt and Hinter.	CR	Rock	CHL	Alpine	0.363 ± 0.077
Hypotrachyna exsecta (Taylor) Hale	FO	Bark	CHL	Temperate	0.339 ± 0.132
Everniastrum cirrhatum (Fr.) Hale ex Sipman	FO	Bark	CHL	Temperate	0.339 ± 0.206
Nephromopsis pallescens (Schaer.) Y.S. Park	FO	Bark	CHL	Temperate	0.324 ± 0.186
Peltigera rufescens (Weiss) Humb.	FO	Rock	CYA	Temperate	0.315 ± 0.143
Acarospora fusca B. de Lesd.	CR	Rock	CHL	Alpine	0.302 ± 0.086
Ramalina roesleri (Hochst. ex Schaer.) Hue	FR	Bark	CHL	Temperate	0.294 ± 0.050
Umbilicaria vellea (L.) Hoffm.	FO	Rock	CHL	Alpine	0.281 ± 0.094
Umbilicaria indica Frey	FO	Rock	CHL	Alpine	0.259 ± 0.118
Aspicilia almorensis Räsänen	CR	Rock	CHL	Temperate	0.256 ± 0.131
Ochrolechia subpallescens Verseghy	CR	Bark	CHL	Temperate	0.254 ± 0.069
Heterodermia dactyliza (Nyl.) Swinscow and Krog	FO	Rock	CHL	Temperate	0.254 ± 0.077
Acarospora fusca B. de Lesd.	CR	Rock	CHL	Alpine	0.233 ± 0.060
Leptogium askotense D.D. Awasthi	FO	Bark	CYA	Temperate	0.220 ± 0.102
Sticta praetextata (Räsänen) D.D. Awasthi	FO	Rock	CHL	Temperate	0.219 ± 0.169
Lecanora fimbriatula Stirt.	CR	Bark	CHL	Temperate	0.202 ± 0.093
Lobaria retigera (Bory) Trevis.	FO	Bark	CYA	Temperate	0.195 ± 0.143
Nephromopsis pallescens (Schaer.) Y.S. Park	FO	Bark	CHL	Temperate	0.181 ± 0.176
Parmeliella philippina (Vain.) P.M. Jørg.	FO	Bark	CYA	Temperate	0.170 ± 0.060
Lecanora himalayae Poelt	FO	Soil	CHL	Alpine	0.155 ± 0.137
Lepraria sp1	CR	Bark	CHL	Temperate	0.153 ± 0.046
Lepraria sp2	CR	Bark	CHL	Temperate	0.146 ± 0.115
Ramalina sinensis Jatta	FR	Bark	CHL	Temperate	0.145 ± 0.073
Chrysothrix chlorina (Ach.) J.R. Laundon	CR	Bark	CHL	Temperate	0.138 ± 0.111
Leptogium burnetiae C.W. Dodge	FO	Rock	CYA	Temperate	0.138 ± 0.078

(*Contd*)

Table 2. (*Contd*)

Lichen taxa	Growth form	Substratum	Photobiont	Distribution	F_v/F_m
Coccocarpia erythroxyli (Spreng.) Swinscow and Krog	FO	Rock	CYA	Alpine	0.135 ± 0.109
Heterodermia dactyliza (Nyl.) Swinscow & Krog	FO	Rock	CHL	Temperate	0.127 ± 0.087
Parmelaria thomsonii (Stirt.) D.D. Awasthi	FO	Bark	CHL	Temperate	0.121 ± 0.167
Lobaria retigera (Bory) Trevis.	FO	Rock	CYA	Temperate	0.116 ± 0.077
Peltigera canina (L.) Willd.	FO	Bark	CYA	Temperate	0.115 ± 0.103
Peltigera canina (L.) Willd.	FO	Rock	CYA	Temperate	0.114 ± 0.115
Leptogium burnetiae C.W. Dodge	FO	Rock	CYA	Temperate	0.110 ± 0.133
Peltigera rufescens (Weiss) Humb.	FO	Rock	CYA	Temperate	0.110 ± 0.069
Lecanora concilianda Vain.	CR	Bark	CHL	Temperate	0.107 ± 0.075
Leptogium burnetiae C.W. Dodge	FO	Bark	CYA	Temperate	0.106 ± 0.086
Parmotrema nilgherrense (Nyl.) Hale	FO	Bark	CHL	Temperate	0.106 ± 0.140
Usnea dendritica Stirt.	FR	Bark	CHL	Temperate	0.090 ± 0.089
Heterodermia diademata (Taylor) D.D. Awasthi	FO	Bark	CHL	Temperate	0.089 ± 0.034
Cetrelia sanguinea (Schaer.) W.L. Culb. and C.F. Culb.	FO	Bark	CHL	Temperate	0.079 ± 0.079
Lobaria retigera (Bory) Trevis.	FO	Rock	BGA	Temperate	0.075 ± 0.088
Heterodermia microphylla (Kurok.) Skorepa	FO	Rock	CHL	Temperate	0.075 ± 0.078
Sticta praetextata (Räsänen) D.D. Awasthi	FO	Bark	CHL	Temperate	0.066 ± 0.033
Ramalina conduplicans Vain.	FR	Bark	CHL	Temperate	0.060 ± 0.108
Graphis subglauconigra Nagarkar and Patw.	CR	Bark	CHL	Temperate	0.043 ± 0.039
Lecanora concilianda Vain.	CR	Bark	CHL	Temperate	0.041 ± 0.041
Myelochroa irrugans (Nyl.) Elix and Hale	FO	Rock	CHL	Temperate	0.041 ± 0.009
Ochrolechia subpallescens Verseghy	CR	Bark	CHL	Temperate	0.023 ± 0.039

Table 3. Mean F_v/F_m of chlorolichen communities

Distribution	Communities	F_v/F_m
Both temperate and alpine	Crustose	0.314 ± 0.158
	Foliose	0.254 ± 0.161
	Fruticose	0.459 ± 0.190
	Bark	0.258 ± 0.178
	Rock	0.363 ± 0.146
	Soil	0.526 ± 0.168
Temperate	Crustose	0.278 ± 0.169
	Foliose	0.251 ± 0.174
	Fruticose	0.386 ± 0.204
	Bark	0.258 ± 0.178
	Rock	0.350 ± 0.178
	Soil	0.536 ± 0.045
Alpine	Crustose	0.389 ± 0.104
	Foliose	0.267 ± 0.090
	Fruticose	0.575 ± 0.083
	Rock	0.379 ± 0.103
	Soil	0.525 ± 0.181

F_v/F_m and lichen communities

F_v/F_m of lichens studied in all the samples ranged from 0.023 (±0.039) to 0.655 (±0.024). *C. subconistea*, a fruticose lichen with terricolous habitat in alpine region had maximum F_v/F_m, whereas *Ochrolechia subpallescens*, a crustose, corticolous lichen in temperate region had the lowest value (Table 2). The mean values of F_v/F_m of lichen communities (all) growing in temperate and alpine regions found to be significantly different ($P = 0.0006$) with alpine lichens having higher mean (0.416 ± 0.159) in comparison to temperate (0.264 ± 0.176). It is obvious that due to the altitudinal difference, both the regions have entirely different climate and hence different in lichen composition[22]. In the present study, a crustose lichen *Aspicilia almorensis* was the only common species encountered in the two localities. Hence, the F_v/F_m of lichen communities were separately compared in both the regions to understand the vitality of individual lichens and their communities in both the regions. The cyanolichens have relatively lower F_v/F_m ranging from 0.075 (*Lobaria retigera*) to 0.315 (*Peltigera rufescens*). Inclusion of F_v/F_m of cyanolichens for statistical analysis substantially influenced values of whole lichen communities. Hence, to obtain similarity while comparing F_v/F_m of lichen communities only the data of chlorolichens are considered (Table 3).

In temperate region, the P value of mean F_v/F_m for all growth-form communities (ANOVA) as well as of each pair (t-test) was found to be insignificant. Whereas in the case of alpine region, the P value (ANOVA) of growth forms significantly differs (Table 4). Similarly, the P value for lichen communities growing on various substrates did not show significant difference in temperate region. In case of alpine region, the mean F_v/F_m value of rock and soil communities was significantly different with soil having higher value (0.525 ± 0.181) and indicating the influence of wet soil to photosynthetic performances of the lichen communities.

Discussion

F_v/F_m is generally considered as an indicator of health of a photosynthetic organism and can be utilized to prepare their vitality index. In healthy, unstressed vascular

Table 4. *P* value of *t*-test and ANOVA for mean F_v/F_m of chlorolichen communities

Distribution	Population	*p* value (*t*-test)	*p* value (ANOVA)
Both temperate and alpine	Temperate vs alpine	0.0039	
	Bark vs rock	0.0158	
	Bark vs soil	0.0004	0.0003
	Rock vs soil	0.0107	
	Crustose vs foliose	0.1883	
	Crustose vs fruticose	0.0072	0.0009
	Foliose vs fruticose	0.0006	
Temperate	Bark vs rock	0.1057	
	Bark vs soil	0.1345	0.1097
	Rock vs soil	0.3283	
	Crustose vs foliose	0.6296	
	Crustose vs fruticose	0.1316	0.1403
	Foliose vs fruticose	0.0657	
Alpine	Rock vs soil	0.0318	
	Crustose vs foliose	0.0680	
	Crustose vs fruticose	0.0017	0.0002
	Foliose vs fruticose	0.0003	

plants[23], the ratio of F_v/F_m comes close to 0.832. Values lower than this will be seen when the plant has been exposed to stress, indicating in particular the phenomenon of photoinhibition[2]. In case of reliably dead material, the value would be <0.1. In normal lichens the values range from 0.6 to 0.76 and sometimes healthy crustose and cyanolichens have more lower values such as 0.5–0.6 (ref. 13). According to these findings, it appears that only six chlorolichens are normal (healthy) which have F_v/F_m value >0.6, as many as 53 lichens are stressed with value ranging from 0.1 to 0.59 and 10 are dead (<0.1). Out of six lichens with F_v/F_m value >0.6, except for *Cladonia sinensis* all the other five samples belongs to the alpine region. Also, all the lichens of the alpine region can be considered alive as they have F_v/F_m value >0.1, however 14 among them are stressed.

The levels of photosynthetic activity in the field were influenced by microenvironmental condition and its response pattern is site- and species-specific[24]. Shade- and sun-exposed lichens may have different photosynthetic performance due to difference in their chlorophyll contents, with shaded ones having more chlorophyll[25]. In case of cyanolichens, it is now well known that the low chlorophyll fluorescence is due to the state of transition in cyanobacteria, where excitation energy in the dark is redistributed to photosystem I at the expense of photosystem II (ref. 26) and due to presence of non-variable phycobilin fluorescence[27]. Therefore, low F_v/F_m values can be utilized to monitor environmental damage, photoinhibition, a marked state transition or high phycobilin content[6].

The stress in the studied area can be related to the availability of water in addition to high intensity light, and to some extent anthropogenic interference. Chlorolichens depend on atmosphere for the nutrient and water; however, the moisture content in the substratum especially in bark and soil certainly contributes to their vitality. We could not collect any data on the moisture content of the substrates and annual variations in the relative humidity of both the regions. There was not much difference between two regions in ambient humidity at the time of sampling. The anthropogenic disturbances in the area include small-scale tourism, adventure sports, grazing and collection of non-timber forest products by local people. The effect of such anthropogenic activities also could not be assessed at the time of lichen collection. It was observed that during mid-day, light intensity of the region is too high (up to 3000 $\mu mol\ m^{-2}\ s^{-1}$) and hence lichens are expected to be active during morning and evening time when the light intensity is reduced. Coxson[28] showed photoinhibition of net photosynthesis following brief exposure to high light and temperature in *Stereocaulon virgatum* and *Stereocaulon tomentosum*. The high intensity of light, apart from causing photoinhibition in lichens also increase the thallus temperature, which further causes loss of water and dryness. Apart from these environmental stress factors, senescence of the lichens leading to low F_v/F_m also cannot be ruled out[6,29]. However, it is difficult to assess the senescence in lichens in field when they are already exposed to stress and unless their growth-related parameters are known. It was observed that the soil, rock and bark in temperate region are much drier while in alpine region, soil and some rocks are wet due to melting of snow and glaciers. The scarcity of water and high intensity of light in temperate region caused severe stress to lichens leading to lower F_v/F_m value. The effects of water stress are probably severe in case of cyanolichens occurring in temperate region, as they require liquid water for the activation of photosynthesis[30]. Their F_v/F_m value ranged from 0.075 to 0.315. It is proposed that in the case of cyanolichens F_v/F_m should be measured after low light treatment instead of darkness[31]. No significant difference between the F_v/F_m of dark adaptation and low

light-treated samples was observed. In alpine region high intensity light caused photoinhibition, however low temperature and wet soil provided water to lichens comparatively for longer duration. Hence, the F_v/F_m range of terricolous lichens in the alpine region ranged from 0.473 to 0.655 with *C. subconistea* having the maximum value.

Lechowicz[32] opined that lichens with quite distinctive photosynthetic response commonly occupy the same habitat. Lechowicz and Adams[33,34] showed that *Cladonia mitis*, *Cladonia rangiferina* and *Cladonia uncialis* occupied distinct microhabitats in Wisconsin Pine Barrens, reflecting the underlying differences in gas exchange characteristics and morphological effect on thallus–water relation. Larson[35] suggested the importance of morphology in controlling the coupling of lichens to its surroundings. Apart from the moisture content in the substratum and atmosphere, growth form may also influence the chlorophyll fluorescence when the F_v/F_m of the community as a whole is considered. Within the growth forms, chlorophyll fluorescence of each species may vary due to the difference in their morphology. The foliose and fruticose lichen communities by having more surface area can be expected to have more photobiont cells and should have better F_v/F_m compared to crustose lichens. However, it is observed that in temperate region the P value of F_v/F_m for all growth-form communities (ANOVA) as well as of each pair (t-test) was found to be insignificant (Table 4), indicating that chlorophyll fluorescence or photosynthetic performance of lichen communities is not influenced by their growth forms. Whereas in the case of alpine region, P value (ANOVA) of growth forms as a whole is significantly different indicating that it influenced the photosynthetic performance of the communities. However, within alpine region when pairs of growth form communities are compared 'crustose–foliose' did not show any significant difference. Similarly, no significant difference was observed in the mean F_v/F_m value of lichen communities growing on different substrata in a temperate region, while in the alpine region lichen communities growing over rock and soil are significantly different with respect to their chlorophyll fluorescence. *Porpidia hydrophila*, though being a crustose lichen and growing on rock in alpine had higher F_v/F_m value (0.616). Likewise, crustose lichen *Buellia indica* growing on rock in the temperate region had higher F_v/F_m (0.552). Fruticose lichens, belonging to the genus *Cladonia* in the alpine region along with their morphological advantages also grow on moist soil, which supports their vitality and enhance their photosynthetic performance. In contrast, *C. sinensis* growing on rock in drier temperate region too had higher F_v/F_m (0.623). These findings indicate that the combinations of morphology, substratum and microenvironment within a given habitat influence the chlorophyll fluorescence of a lichen rather than any one parameter acting individually. To understand the exact role of growth forms and substratum towards photosynthetic performance of lichen community as a whole, an entirely different set of experiment and statistical analysis are needed with sufficient representation of samples under both the categories. Apart from moisture in substratum and environment, another factor that may influence the vitality of lichens is the bryophytes (moss and leafy liverworts) which prefer the same microclimate and grow in association with lichens[36]. The mosses are known for their water holding ability and lichens growing along with them may directly or indirectly get benefitted by such an association. That may be one of the reasons why some lichens such as *C. ceratophyllina*, *C. scabriuscula* and *C. sinensis* though having saxicolous habitat in temperate region, have better F_v/F_m value. It would be interesting to study such association as a strategy for survival during drought.

Sometimes, it may also happen that some crustose lichens with crowded apothecia may yield low chlorophyll fluorescence, because, except thalline margins major portion of apothecia do not have algal cells. In case of lecidioid, lichens, F_v/F_m may go further low as they lack algal cells even in apothecial margin and apothecia are usually black. Hence, care has to be taken while interpreting the fluorescence value of such lichens. *Lecanora concilianda* is one such lichen with crowded, black apothecial and thalline margin, yielded low fluorescence (0.107), otherwise appearing healthy.

Conclusion

Net photosynthesis in lichens is controlled by photon flux density, tissue temperature and tissue water content. Each lichen population has a characteristic seasonal pattern of photosynthetic response to these controlling variables[7,32]. As mentioned earlier, the study was carried out during early summer when lichens of the region have already started experiencing stress. Monitoring chlorophyll fluorescence of lichens throughout the year at regular intervals may indicate the best season for all the lichens to be at their full vigour. However based on F_v/F_m, lichens can be classified into three categories, viz. normal (healthy), moderately stressed and severely stressed with F_v/F_m range 0.5–0.76, 0.3–0.49 and 0.1–0.29 respectively. It may not be a good idea to consider lichens having values below <0.1 as dead unless their value consistently goes down to zero. The lichens are poikilohydric, can survive severe bouts of desiccation and they rejuvenate once the favourable condition returns. Hence, it is difficult to declare them as dead but can be included under severely stressed category.

The present study, though preliminary, found to be useful in assessing the vitality of lichens growing in a given area. The study paves path for an in-depth study on several related aspects such as physiological advantages for lichens by having different shapes, size and structure

(growth form), selecting varied substratum for colonization and developing association with other smaller plants (say bryophytes). Lichens are cold and desiccation-tolerant organisms and their photo-inhibition mechanism is another interesting aspect to study as they luxuriantly grow in the regions having high light intensity such as cold desert of Himalayas and Antarctica. It is clear from our study that along with classical studies such as lichen taxonomy or floristics, there is also an urgent need for observational and experimental studies in India.

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