Chlorophyll a fluorescence measurements for validating the tolerant bryophytes for heavy metal (Pb) biomapping

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Chlorophyll a fluorescence measurement has been used as a probe to examine and compare the tolerance of bryophytes against heavy metal stress caused in field trials and under laboratory conditions. The ratio of variable fluorescence (Fv) to the maximal fluorescence (Fm) in dark-adapted leaves termed as ‘maximal photochemical efficiency’ of PS II (Fv/Fm) was measured in selected bryophytes to study their quantum efficiency. Tolerance potential for lead (Pb) in bryophytes was evaluated statistically by using Dunkan’s Multiple Range Test, which indicates that lead-treated moss Grimmia anodon and liverwort Riccardia pinguis exhibited the most physiological damage of PS II. Conversely, minimal changes were observed in Bar-bula vinealis and Thuidium cymbifolium. Field trials of all the selected bryophytes exhibited moderate changes in the Fv/Fm ratio except G. anodon and R. pinguis. This variation in susceptibility is due to stress caused by metal pollution. Therefore, it is desirable to study the relative tolerance potential of bryophytes as little is known about their susceptibility to metals. Validated tolerant bryophytes species will have multiple applications in studies of biomapping, forest enrichment and carbon gain.

Keywords: Bryophytes, chlorophyll fluorescence, heavy metal, Pb tolerance.

HEAVY metals are widespread as environmental pollutants resulting from human activities. They are accumulated by some bryophytes without diminution of growth. An evaluation of bryophyte health is therefore required to assess their response to metal pollutants to validate tolerance. Bryophytes are widely used as biomonitors of atmospheric metal deposition¹-⁴ because of their ability to accumulate elevated concentrations of metals without impairing physiological parameters. Bryophytes are also known to suffer a decrease of chlorophyll content especially at low pH⁵, thus affecting photosynthetic pigment concentrations and ultimately the vitality of the species depending upon their tolerance. However, this character-

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Table 1. Moss and liverwort species measured for chlorophyll a fluorescence ($F_o / F_m$) at different sites (rural and urban) of Mukteshwar for natural stress

<table>
<thead>
<tr>
<th>Moss species</th>
<th>Forest cover</th>
<th>Forest road side</th>
<th>Urban site 2 km</th>
<th>Urban site 1 km</th>
<th>Bus stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbula</td>
<td>0.803 ± 0.006*</td>
<td>0.800 ± 0.011*</td>
<td>0.738 ± 0.031</td>
<td>0.689 ± 0.010</td>
<td>0.594 ± 0.013</td>
</tr>
<tr>
<td>Thuidium</td>
<td>0.811 ± 0.021*</td>
<td>0.791 ± 0.006*</td>
<td>0.725 ± 0.021</td>
<td>0.667 ± 0.031</td>
<td>0.584 ± 0.007</td>
</tr>
<tr>
<td>Plagiothecium</td>
<td>0.779 ± 0.015*</td>
<td>0.769 ± 0.023*</td>
<td>0.734 ± 0.006*</td>
<td>0.670 ± 0.022</td>
<td>0.542 ± 0.027</td>
</tr>
<tr>
<td>Polytrichum</td>
<td>0.831 ± 0.008</td>
<td>0.773 ± 0.021</td>
<td>0.705 ± 0.021</td>
<td>0.624 ± 0.020</td>
<td>0.572 ± 0.009</td>
</tr>
<tr>
<td>Dicranum</td>
<td>0.789 ± 0.005*</td>
<td>0.756 ± 0.004*</td>
<td>0.693 ± 0.011</td>
<td>0.632 ± 0.007*</td>
<td>0.547 ± 0.004*</td>
</tr>
<tr>
<td>Bryum</td>
<td>0.813 ± 0.032</td>
<td>0.795 ± 0.032</td>
<td>0.687 ± 0.004</td>
<td>0.644 ± 0.011</td>
<td>0.522 ± 0.031</td>
</tr>
<tr>
<td>Hylomnium</td>
<td>0.775 ± 0.007*</td>
<td>0.764 ± 0.003*</td>
<td>0.680 ± 0.007</td>
<td>0.653 ± 0.030</td>
<td>0.537 ± 0.005</td>
</tr>
<tr>
<td>Grimmia</td>
<td>0.790 ± 0.021</td>
<td>0.698 ± 0.013</td>
<td>0.575 ± 0.020</td>
<td>0.300 ± 0.010</td>
<td>0.061 ± 0.008</td>
</tr>
<tr>
<td>Ricordia</td>
<td>0.762 ± 0.009</td>
<td>0.531 ± 0.006</td>
<td>0.401 ± 0.011</td>
<td>0.204 ± 0.004</td>
<td>0.099 ± 0.017</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SE.
Significance test of ANOVA and DMRT has been done at 1% and 5% significance level.
Values superscripted with the same alphabets in horizontal row are not significantly different at 1% and 5% significance level at different sites.
*Values in horizontal row are significantly different at 5% significance level.

Figure 1. Comparative graph of percentage decrease in $F_o / F_m$ ratio of different bryophytes at maximum concentration (200 mM) over control and bus stand over Mukteshwar forest cover.

(maximum 3000 μmol m⁻² s⁻¹) during the measurement. At the completion of measurement (1 s) the calculated parameters of $F_o$, $F_v$, $F_m$, $F_o / F_m$ and area above the curve were automatically displayed and stored to instrument memory.

Similar sized specimens of bryophytes collected from natural habitats were brought to the laboratory within 24 h and kept in petri dishes. Control and treatments of lead acetate (5, 10, 100 and 200 mM) were applied in a closed chamber (45 x 66 cm) and CF was measured after a 30 min dark adaptation.

Samples were analysed in triplicates to conduct statistical analysis. Values were represented as mean ± standard error. ANOVA revealed significant differences in the $F_o / F_m$ values of the bryophytes in different concentrations (for $P ≤ 0.01$, $P ≤ 0.05$) utilizing Dunn’s Multiple Range Test. The results are presented on the basis of statistical variability in bryophytes tolerance potential with respect to heavy metal concentrations.

Measurement of $F_o / F_m$ ratio data remained nearly constant in all the selected bryophytes growing under forest cover (Table 1) in native conditions. Many of these figures fall within a close range, i.e. 0.831–0.700 for bryophyte species under forest cover and road side areas. By comparison, marginally lower values were measured for bryophytes growing in urban sites (1 and 2 km) and in proximity to the bus stand. However, $F_o / F_m$ values of bryophytes growing under forest cover and close to the bus stand exhibited significantly different ($P ≤ 0.01$, 0.05) results in all the studied mosses and liverworts, which confirms the effects of response to different kinds of environmental stresses on photosynthetic functions. Data as a whole did not exhibit lower $F_o / F_m$ values in most of the bryophytes except G. anodon and R. pinguis which exhibited minimum values as 0.061 and 0.099 respectively, growing near the bus stand (Figure 1).

An attempt was made to understand the effect of Pb on the photosynthetic apparatus status of bryophytes. It was observed that with the increase in concentration of heavy metal (Pb), the $F_o / F_m$ ratio decreased linearly (Table 2). CF remained nearly constant in most of the moss species treated by 5 mM of Pb solution. The decrease in the ratio was maximal in G. anodon (91.8%) and R. pinguis (89.3%), whereas the minimum was in Barbula vinaulis (25.1%) and T. cymbiformis (25.4%) in 200 mM concentrations over control (Figure 1). Non-significant ($P ≤ 0.01$, 0.05) results were observed in some bryophytes up to 50 mM lead concentration, whereas the same was significantly different and more pronounced at 5% at 100 mM in T. cymbiformis. The inhibition rate of photosynthesis was measured by feeding different concentrations of Pb to G. anodon and R. pinguis.

In forest cover high $F_o / F_m$ ratios reflect that under undisturbed and healthy habitats, plants undergo normal physiological functions in both tolerant and sensitive moss species. The $F_o / F_m$ ratio in natural habitats ranged between 0.831 and 0.700 in forest cover; there were substantial differences in $F_o$ over the area of hydrated bryophytes that could be considered as striking species characteristics. The same was found minimum in moss.
collected near the bus stand. It confirms that environmental stress parameters had an adverse effect and the same was suggested by the increase in fluorescence. The main sources of stress at urban sites and near the bus stand were derived from increasing vehicular traffic, tourist activity, leaded batteries, wear and tear of tyres and motor parts. Pollutant discharge from local sources in densely areas of city also plays a major role in the increase of metals resulting in pollutant load that could diminish the absorption characteristics and consequently, CF. Stress by pollutants including metal precipitation could be the cause of inhibition of PS II efficiency in some species which can be identified as sensitive species. Therefore, the present findings are in agreement with the described adverse effects of metals on CF parameters. Since the physiological state of the photosynthetic apparatus differed at different tested sites (forest cover, forest road side, urban sites and bus stands), we believe that, the heterogeneity of CF may reflect the quality of the growth zone.

It was evident that the bryophyte behaviour at different locations may be due to atmospheric influence. However, values which range from 0.8 to 0.7 show nearly identical behaviour, and indicate a similar physiological state of the photosynthetic apparatus and a favourable habitat. By contrast, the $F_{v}/F_{m}$ value of $G.\text{anodon}$ and $R.\text{pinguis}$ quickly changed upon treatment and was lower even upon a slight change of location.

Using $F_{v}/F_{m}$ values as a screening parameter, it was possible to distinguish stress induced by Pb within 30 min thus, to identify the tolerant species or status of photosynthetic efficiency. Pb has a strong effect on chloroplasts but most of the Pb is sequestered as phosphate precipitation or bound to the cell wall and is responsible for membrane damage and leakage in bryophytes. $F_{v}/F_{m}$ ratio remained nearly constant in most of the moss species at lower concentration (5 mM), revealing that the maximal photochemical capacity of the photosynthetic cells remains unchanged at lower concentration.

PS II activity (presented by $F_{v}/F_{m}$) was observed to decrease due to Pb toxicity, which indicates that this metal strongly intervenes in photosynthetic electron transport. When metal concentration was increased to 50 mM, the $F_{v}/F_{m}$ values start to decrease, whereas the decrement was more pronounced at 100 and 200 mM concentration. Present results affirmed that $G.\text{anodon}$ and $R.\text{pinguis}$ seem to be the least tolerant to metal stress of the studied bryophytes as evidenced by the high fluorescence signal, i.e. lower $F_{v}/F_{m}$ values (Table 2). However, $B.\text{vinealis}$, $T.\text{cymbifolium}$ and $B.\text{cellulare}$ are comparatively more tolerant bryophyte species and are recommended for biomass as well as use in forest enrichment.

The present results suggest multiple applications of CF which can be applied as a useful tool to follow the response of bryophytes to environmental factors and also in validating tolerant species.

**Table 2.** Moss and liverwort species validated for Pb tolerance by measuring chlorophyll $a$ fluorescence ($F_{v}/F_{m}$) in laboratory after 30 min treatment for metal stress

<table>
<thead>
<tr>
<th>Moss species</th>
<th>Control</th>
<th>5 mM</th>
<th>50 mM</th>
<th>100 mM</th>
<th>200 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbula</td>
<td>0.812 ± 0.040*</td>
<td>0.810 ± 0.011*</td>
<td>0.764 ± 0.020</td>
<td>0.679 ± 0.005</td>
<td>0.608 ± 0.016</td>
</tr>
<tr>
<td>Thuidium</td>
<td>0.803 ± 0.004*</td>
<td>0.799 ± 0.021 b4a8</td>
<td>0.775 ± 0.007*</td>
<td>0.711 ± 0.011*</td>
<td>0.599 ± 0.022</td>
</tr>
<tr>
<td>Plagiochasma</td>
<td>0.809 ± 0.007</td>
<td>0.781 ± 0.043</td>
<td>0.746 ± 0.011</td>
<td>0.630 ± 0.005</td>
<td>0.502 ± 0.004</td>
</tr>
<tr>
<td>Polyticum</td>
<td>0.710 ± 0.016</td>
<td>0.640 ± 0.024</td>
<td>0.605 ± 0.005</td>
<td>0.531 ± 0.006</td>
<td>0.490 ± 0.020</td>
</tr>
<tr>
<td>Dicranum</td>
<td>0.749 ± 0.010</td>
<td>0.713 ± 0.006</td>
<td>0.610 ± 0.014*</td>
<td>0.609 ± 0.021a*</td>
<td>0.510 ± 0.014</td>
</tr>
<tr>
<td>Brynum</td>
<td>0.815 ± 0.012*</td>
<td>0.808 ± 0.014a*</td>
<td>0.602 ± 0.004</td>
<td>0.540 ± 0.006</td>
<td>0.501 ± 0.007</td>
</tr>
<tr>
<td>Hlyocodium</td>
<td>0.794 ± 0.003</td>
<td>0.771 ± 0.008</td>
<td>0.705 ± 0.021</td>
<td>0.601 ± 0.018</td>
<td>0.509 ± 0.002</td>
</tr>
<tr>
<td>Grimmia</td>
<td>0.750 ± 0.040</td>
<td>0.610 ± 0.012</td>
<td>0.401 ± 0.016</td>
<td>0.204 ± 0.020</td>
<td>0.061 ± 0.011</td>
</tr>
<tr>
<td>Riccardia</td>
<td>0.741 ± 0.032</td>
<td>0.563 ± 0.008</td>
<td>0.479 ± 0.011</td>
<td>0.031 ± 0.014</td>
<td>0.079 ± 0.017</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SE.
Significance test of ANOVA and DMR has been done at 1% and 5% significance level.
Values superscripted with the same alphabets in horizontal row are not significantly different at 1% and 5% significance level at different sites.
*Values in horizontal row are significantly different at 5% significance level.


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Unique graft combination of tea, Cr-6017/UPASI-9

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A unique graft combination in tea comprising a quality clone Cr-6017 as a scion and drought hardy high-yielding clone UPASI-9 as root stock was studied under commercial conditions. Average survival rate of cuttings of grafted plants in the nursery was above 85%. Grafted plants of Cr-6017/UPASI-9 were more vigorous and irrespective of the year from planting registered significantly high yields over ungrafted controls. Grafting resulted in increase in the number of branches, branch length and reduction in the branch angle. Grafted plants registered significantly low values of mesomerophy index, vulnerability index, shoot water potential and high values of F₀/Fm. Grafted and ungrafted Cr-6017 plants registered significantly high quantities of total polyphenols and catechins. Variation in the biochemical composition of green leaf and made tea of grafted and control plants of Cr-6017 was statistically non-significant. Quality attributes of teas manufactured from Cr-6017 were superior compared to teas from UPASI-9.

Keywords: Anatomy, biochemical composition, bush architecture, Cr-6017/UPASI-9, drought tolerance, nursery grafting.

GRAFTING has been practised for a long time all around the world. Plantation crops like rubber and horticultural crops like apple, pear, plum, and other woody perennials received most attention in this aspect. In the case of tea, until about 50 years ago, seeds were the only source of propagation. In the absence of suitable mode of vegetative propagation, grafting was tried in the beginning of the last century by certain tea-growing countries to propagate the selected clones primarily for the production of seeds. In the early thirties, a simple and economic method of vegetative propagation, in which capacity to root and rapidly form satisfactory nursery plants from single nodal cuttings from the young shoots was demonstrated. Although it took some time to standardize the practice, the method has been widely used since 1950s by most of the tea-growing countries of the world.

Earlier work on grafting in tea mainly aimed at induction of more number of flowers in a shorter time for the production of seeds. In the case of budding and grafting methods followed in tea, the rootstock had been either established in the field or a seedling from the nursery. During 1971, a unique method was demonstrated for cleft

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