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SHORT COMMUNICATION

QUALITATIVE AND QUANTITATIVE EFFECT OF LIGHT ON LEAF SENESCENCE IN A SUBMERGED AQUATIC WEED, *HYDRILLA VERTICILLATA*

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Incubation of isolated leaves of *Hydrilla verticillata* (L.F.) Royle, a submerged aquatic weed, under different qualities of light given in a daily cycle showed a faster decline in chlorophyll and protein contents under red light, while a slower decline of the same under far-red and blue light than dark control. When the photon fluence rate of daily light cycle was varied (0 – 62.5 µmol m^{-2} s^{-1}), senescence-induced loss of chlorophyll and protein was found to be fluence rate-dependent suggesting a quantitative role for light. Changing duration of daily photoperiod (0 – 24 h), instead of fluence rate, also had a quantitative effect on senescence, particularly chlorophyll loss. In another experiment, where light was given in different combinations of photon fluence rate and duration (in daily cycle) having total fluence (fluence rate x duration) constant, chlorophyll loss was almost fluence-dependent except when the duration was too long (20 h). However, senescence-induced protein loss was greater at higher photon fluence rates compared to dark control.

***Key words*: Chlorophyll, *Hydrilla*, photon fluence, photoperiod, protein, senescence.**

Senescence is a developmental process, which is tightly regulated by age as well as environmental factors (Yoshida *et al.* 2001). Recently it is reported that darkness induces symptoms of senescence by expressing some of the senescence-associated genes (SAGs) in dark-adapted leaves (Fujiki *et al*. 2001). Light generally delays leaf senescence (Thimann *et al.* 1977, Kar and Feierabend 1984). However, in certain cases, light has been reported to accelerate senescence as revealed from chlorophyll loss (Sayeed *et al*. 1985). Such light-induced chlorophyll loss has also been demonstrated in the leaves of submerged aquatic plants including *Hydrilla verticillata* (Kar and Choudhuri 1987, Begum and Choudhuri 1993). Regarding regulation of senescence by light, there are variable reports on both involvement of phytochrome (Biswal and Biswal 1984) and regulation by photophosphorylation (Thimann *et al*. 1977) depending on the nature of the effect of light. In the present investigation, attempt has been made to characterize the effect of light, both quantitative and qualitative, on senescence of isolated leaves of *Hydrilla verticillata*, which belongs to a submerged aquatic habitat where light is attenuated both in terms of intensity and quality.

Healthy mature twigs of *Hydrilla verticillata* (L.f.) Royle were collected and leaves from subapical portions (3-4 cm behind the tips) were isolated. Weighed amounts (100 mg for each replicate) of randomized leaf samples were floated on 40 ml distilled water in petridishes and subsequently incubated either in continuous darkness or under specified light condition. In one experiment, light of different quality (red, far-red and blue), produced from two cool fluorescent tubes following the technique suggested by Chawan et al. (1971), was given at fixed photon fluence rate (31.25 µmol m^{-2} s^{-1}) in daily cycle of 8 h light/16 h darkness. In another experiment, light of

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varied fluence rate (0, 12.5, 25, 37.5 and 62.5 µmol m^{-2} s^{-1}) was given (by manipulating the distance between light source and sample) in a daily cycle. In case of third experiment, light of fixed fluence rate (31.25 µmol m^{-2} s^{-1}) was given for different durations (0, 6, 8, 10 12, 14, 16 and 24 h) in daily cycle. In the last experiment, leaves were incubated under conditions of varied fluence rate and duration keeping the total fluence fixed (fluence rate x duration = fluence; 12.5 µmol m^{-2} s^{-1} x 20 h, 25 µmol m^{-2} s^{-1} x 10 h, 50 µmol m^{-2} s^{-1} x 5 h, 75 µmol m^{-2} s^{-1} x 3.3 h, 100 µmol m^{-2} s^{-1} x 2.5 h and 125 µmol m^{-2} s^{-1} x 2 h). Incubation was done in a temperature-controlled (25 ± 2 ºC) growth room and at intervals or at the end of incubation leaves were analyzed for the contents of total chlorophyll, following the method of Arnon (1949), and total protein, according to Lowry *et al*. (1951). Each experiment was repeated thrice with three replicates each time and the mean values were used to calculate the results. Standard errors around means, calculated following the method of Clarke (1969), were shown in the figures as bars.

Isolated leaves of *Hydrilla* kept under different light qualities and darkness showed a faster decline in the content of both total chlorophyll (Fig. 1A) and total protein (Fig. 1B) during incubation under red light, while such decline was slower under far-red and blue light than in dark control. It appears that light has a qualitative role on the process of senescence mediated by phytochrome, although photodynamic effect is more likely to be involved as can be revealed from subsequent observations. Similar type of light-induced chlorophyll loss in senescing wheat leaves was claimed to be independent of phytochrome (Sayeed *et al*. 1985). When the leaves were incubated under different photon fluence rates, both chlorophyll and protein contents decreased proportionately faster with increasing fluence rate (Fig. 2A and B), indicating the possible involvement of photo-oxidative damage during senescence in light, as was explained earlier (Kar and Choudhuri 1987). Increasing photon fluence rate probably caused higher accumulation of peroxides and free radicals through faster electron transport in chloroplasts. Supports come from our earlier observation that continuous irradiance of *Hydrilla* leaves resulted in a faster chlorophyll loss that can be prevented largely by treating the leaves with DCMU, a PS II electron transport inhibitor (Kar and

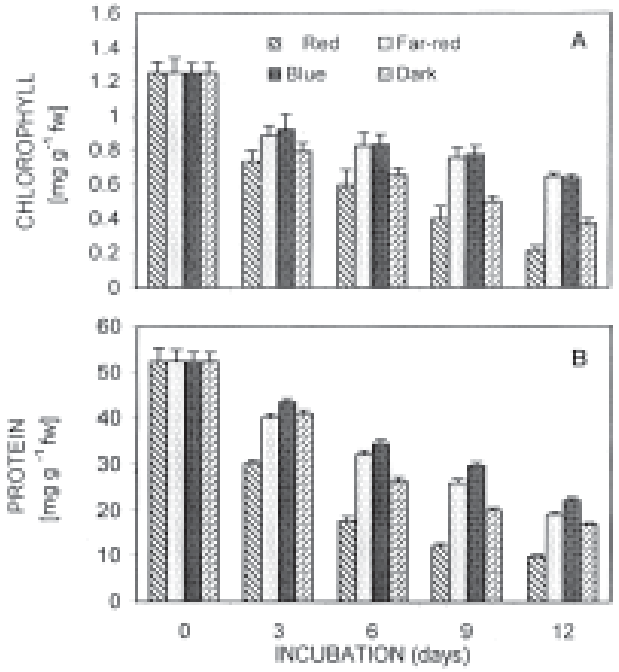

Fig. 1. Changes in the contents of chlorophyll (A) and protein (B) in isolated leaves of *Hydrilla verticillata* (L.f.) Royle during senescence under different qualities of light (red, far-red and blue) given in daily cycle (8 h L/ 16 h D) for 12 days.

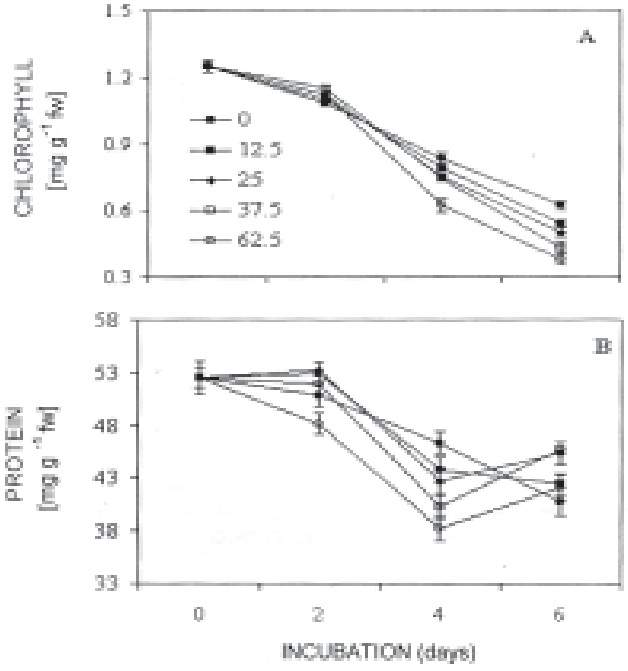

Fig. 2. Changes in the contents of chlorophyll (A) and protein (B) in isolated leaves of *Hydrilla verticillata* (L.f.) Royle during senescence under different photon fluence rates (µmol m^{-2} s^{-1}) of light given in daily cycle (8 h L/ 16 h D) for 6 days.

Choudhuri 1987). Interestingly, in case of leaf senescence, where light was found to retard both chlorophyll and protein loss, DCMU was without effect (Thimann *et al*. 1977), although Fujiki *et al*. (2001) demonstrated that DCMU treatment causes the expression of dark-inducible genes (those normally expressing in senescing leaves) under illumination. When the duration of daily light was varied at fixed fluence rate (31.25 µmol m^{-2} s^{-1}), effect of light was different for chlorophyll and protein loss (Fig. 3A and B). Chlorophyll loss was higher at longer durations of daily light exposure (more than 12 h) than the dark control, while the same was lower at shorter durations (below 12 h). Moreover, chlorophyll loss showed a positive correlation with the duration of light once again indicating a quantitative photodynamic effect of light on particularly chlorophyll degradation. On the other hand, protein loss was faster at daily light durations either too long or too short. Although this behaviour of protein changes cannot be explained by photodynamic effect of light, it is clear that changes in chlorophyll content during senescence may not always be associated with a similar change in protein level. Interestingly, when light was given, a combination of varied fluence rate and daily durations keeping the total fluence constant, chlorophyll loss was apparently fluence-dependent (Fig. 4A) and followed reciprocity law up to certain duration of daily light (10 h), although here light rather retarded chlorophyll loss compared to dark control. However, when duration was 20 h, light caused faster degradation of chlorophyll even though the total fluence remained same. On the other hand, protein loss (Fig. 4B) was slightly higher than that of dark control when daily light was given at lower fluence rate for longer duration, but the decline was significantly greater than the dark control, when light was given at higher fluence rate for shorter duration (100 µmol m^{-2} s^{-1} x 2.5 h and 125 µmol m^{-2} s^{-1} x 2 h).

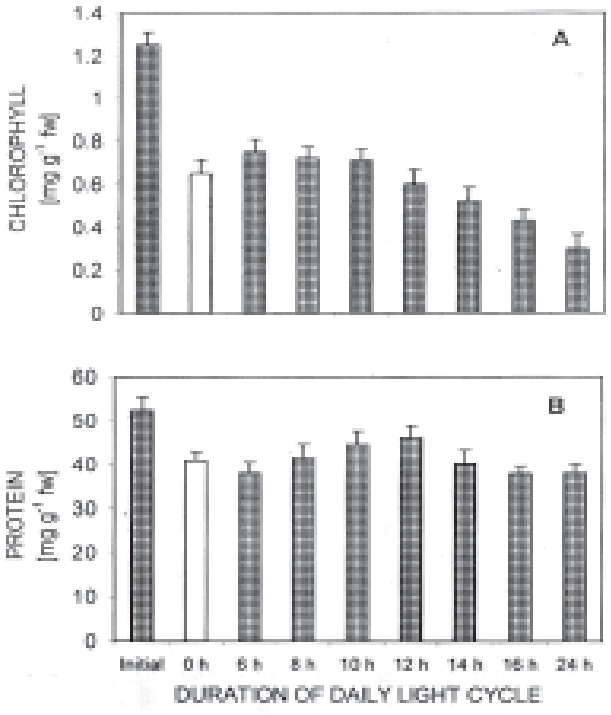

Fig. 3. Changes in the contents of chlorophyll (A) and protein (B) in isolated leaves of *Hydrilla verticillata* (L.f.) Royle after 6 days senescence under daily cycle of light for diffrent durations.

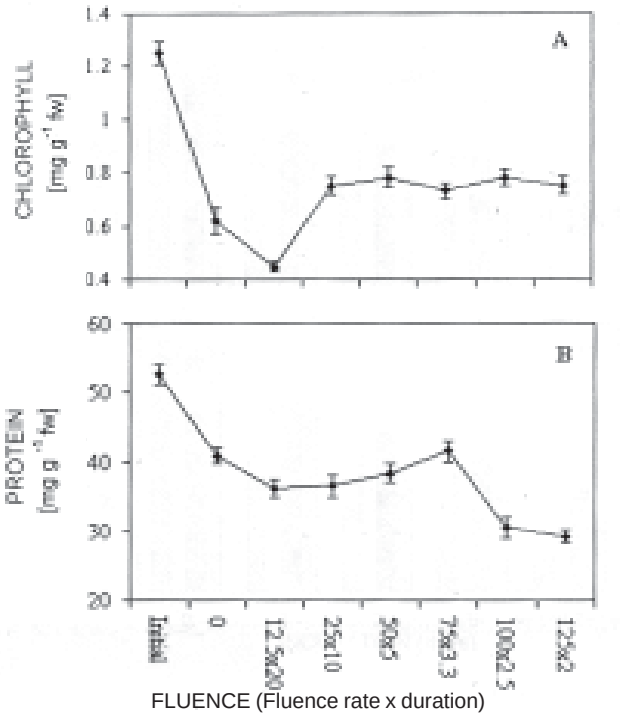

Fig. 4. Changes in the contents of chlorophyll (A) and protein (B) in isolated leaves of *Hydrilla verticillata* (L.f.) Royle after 6 days senescence under diffrent combinations of photon fluance rate and duration in daily cycle (8 h L/ 16 h D) having total fluance (fluance rate x duration) constant.

It may be concluded that the light-induced chlorophyll loss during senescence of *Hydrilla* leaves is dependent on fluence (probably due to photooxidative degradation by the photosynthetically generated free radicals and peroxides), although the role of a photoperiodically regulated component cannot be ruled out, as red/far-red

light showed opposite effects on chlorophyll and protein loss during senescence. Changes in the proteins of the leaves during light incubation is far more complicated, but need not follow the pattern of changes showed by chlorophyll level.

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