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Characterization of Hill Activity of A Submersed Aquatic Angiosperm (Sago Pondweed)

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ABSTRACT

The Hill activity of isolated chloroplasts from mature leaves (40 to 50 days old) of sago pondweed (*Potamogeton pectinatus* L.), a submersed aquatic macrophyte, was characterized with respect to light intensity, temperature, pH, Mg^{2+} ions and reaction time. A maximum Hill activity of 45 μmole DCIP red/mg chl.h was observed at a light intensity of 60 $\mu E/m^2 \cdot sec$, temperature 20C, pH 7.5, and with addition of Mg^{2+} at a concentration of 3 mM during the isolation of chloroplasts and also in the reaction mixture incubated for 10 min. Further increase in any one of the above criteria had an adverse effect on Hill activity. Hill activity was several-fold higher in spinach (*Spinacia oleracea* L.), a terrestrial angiosperm, than in sago pondweed and spinach required a much higher light intensity (240 $\mu E/m^2 \cdot sec$). A gradual decline in the Hill activity with increase in leaf age in both sago pondweed and spinach was noted. Such an age effect was also observed in induced-ageing experiments, and it was concluded that the Hill activity undergoes a similar deterioration in submersed aquatic and terrestrial plants.

INTRODUCTION

Submersed aquatic macrophytes are an acute weed problem in some parts of the world (4, 9, 19, 20). The reason for the rapid spread and dominance of certain submersed plants in the aquatic environments is often ascribed to their growth and physiological behaviour (3, 10, 11, 19, 21, 22). In recent years, several attempts have been made to analyse the various physiological processes of some submersed aquatic angiosperms in order to find better control measures. A detail study of the physiological characteristics of the sago pondweed (*Potamogeton pectinatus* L.), a submersed aquatic angiosperm which invades the ponds and lakes of the tropics and subtropics, is lacking in the literature. Recently, Jana and Choudhuri (11) reported that sago pondweed had higher rates of net photosynthesis, photorespiration and respiration in comparison with hydrilla (*Hydrilla verticillata* (L.F.) Royle) and vallisneria (*Vallisneria spiralis* L.). The high rate of photorespiration in sago pondweed was shown to be responsible for lowering its apparent photosynthesis. Measurement of the apparent rate of photosynthesis is, therefore, always interfered with photorespiration in this species. Thus the study of the Hill activity, which is an important component of photosynthesis signifying the functional status of chloroplasts, will eliminate the possibility of such interference by photorespiratory activity in sago pondweed. While the Hill activity was adequately studied in characterizing the photosynthetic capacity of a number of land plants, such studies with respect to submersed aquatic plants are scarce in the literature. Hence, this investigation was undertaken to study the Hill activity in sago pondweed, and to characterize the effects of various factors which are known to exert influence on the Hill activity of chloroplasts isolated from leaves of several land plants. It was also thought worthwhile to study the effect of ageing on the Hill activity of sago pondweed in comparison with that of a terrestrial plant, since it was shown that the degradation of chloroplasts occurred during senescence of leaves of a number of land plants (5, 8, 17). With these objectives in mind the present study was undertaken.

METHODS AND MATERIALS

Sago pondweed plants were collected from the adjoining ponds and lakes close to the University of Burdwan, Burdwan, India and spinach plants were collected from the University Crop Research Farm.

Chloroplasts were isolated from mature leaves (40 to 50 days old) of sago pondweed and spinach by the method of Nolan and Smillie (15) with the following modifications. Chloroplasts were prepared by gentle homogenization of leaves in a mortar at 0 to 4 C in a medium containing 50 mM sodium phosphate buffer (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$ and 0.5% (w/v) BSA. Mercaptoethanol at 5 mM was also included in the medium used for isolating chloroplasts but was omitted from the reaction mixture.

Chlorophyll content of the chloroplast suspension was measured according to Arnon (1).

Unless otherwise stated, the Hill activity of isolated chloroplasts was determined by the photoreduction of 2,6-dichloroindophenol (DCIP) measured at 620 nm (12) in a Spectrochem Spectrophotometer following a 10 min illumination at a light intensity of 60 $\mu E/m^2 \cdot sec$. The reaction mixture (10 ml) contained: 45 mM sodium phosphate buffer (pH 7.5), 45 mM NaCl, 3 mM $MgCl_2$, 0.012 mM DCIP, 0.045% (w/v) BSA and about 20 μg chlorophyll. All reactions were carried out in air at 20 C. In some experiments, the Hill activity of spinach leaves was determined in the same manner with the exception that the light intensity was increased to 240 $\mu E/m^2 \cdot sec$.

Effects of different environmental factors like light intensity, temperature and pH on the Hill activity were studied by keeping all the variables constant except that under study. To examine the effect of Mg^{2+} on the Hill activity, experiments were performed in three ways: Different concentrations of Mg^{2+} were used during isolation of chloroplasts, and adhering Mg^{2+} was removed from chloroplasts by washing several times with the buffer, but the assay mixture remained without Mg^{2+}; in another, the isolation of chloroplasts was made without Mg^{2+} but the assay mixture contained different concentrations of Mg^{2+}; while in the third set of experiments, Mg^{2+} (3 mM) was used during isolation of chloroplasts and the reaction mixture contained different concentrations of Mg^{2+}. In the control set, Mg^{2+} was added neither in the isolation medium nor in the reaction mixture.

The effect of ageing on the Hill activity was determined using two methods. In one method, leaves of three different age groups (young: 20 to 30 days, mature: 40 to 50 days, and old: 60 to 70 days of age) were utilized. In the other method, the Hill activity was determined in isolated mature leaves with increasing dark incubation time at 22 ± 1 C in water (induced-ageing) containing 25 μg/ml streptomycin sulphate added to prevent bacterial growth. All experiments reported here were performed during the winter season.

Each experiment was replicated six times and the data included in the tables were statistically analyzed by Duncan's Multiple Range Test (7) at 95% confidence level. Standard errors (SE) around the mean were also calculated (7) and plotted in the figures.

RESULTS AND DISCUSSION

The effect of light intensity on the Hill activity of mature leaves (40 to 50 days old) of sago pondweed is shown in Figure 1. The Hill activity gradually increased with increasing light intensity, reached a peak at 60 $\mu E/m^2 \cdot sec$ and declined thereafter. The Hill activity is, however, negligible at the light intensity of 100 $\mu E/m^2 \cdot sec$. Available reports (12, 15) and the data reported here for the spinach chloroplasts (Table 2) indicate that the optimum requirement of light intensity for the Hill activity of chloroplasts isolated from land plants is several-fold higher than that of sago pondweed. This is obviously due to the adaptation of a submersed aquatic plant to the prevailing light conditions during its growth.

The effect of temperature on the Hill activity was next studied at the light intensity of 60 $\mu E/m^2 \cdot sec$. It is clear from Figure 2 that the Hill activity in sago pondweed increased with temperature up to 20 C and then sharply declined. The Hill activity was completely halted at 35 C. The low temperature tolerance of the Hill activity of sago pondweed might be associated with its low temperature resistant nature adapted to growing conditions. High temperature injury of Hill activity is probably due to temperature-induced deterioration of the chloroplast membrane (13, 14, 15). One of the reasons for optimum growth of this plant during the winter months and its overall senescence with the advent of the summer season in the tropics and subtropics might be associated with the

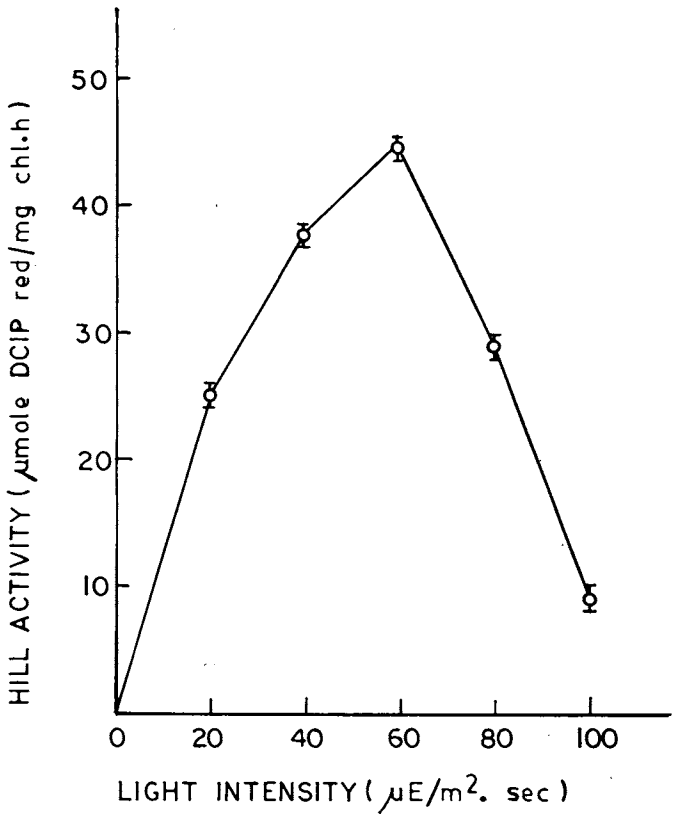

Figure 1. Effect of light intensity on Hill activity (μmole DCIP red/mg chl. h) of mature leaves (40 to 50 days old) of sago pondweed at pH 7.5 and temperature 20 C. The vertical bars indicate the standard errors of the means. Each point is the mean of six replications.

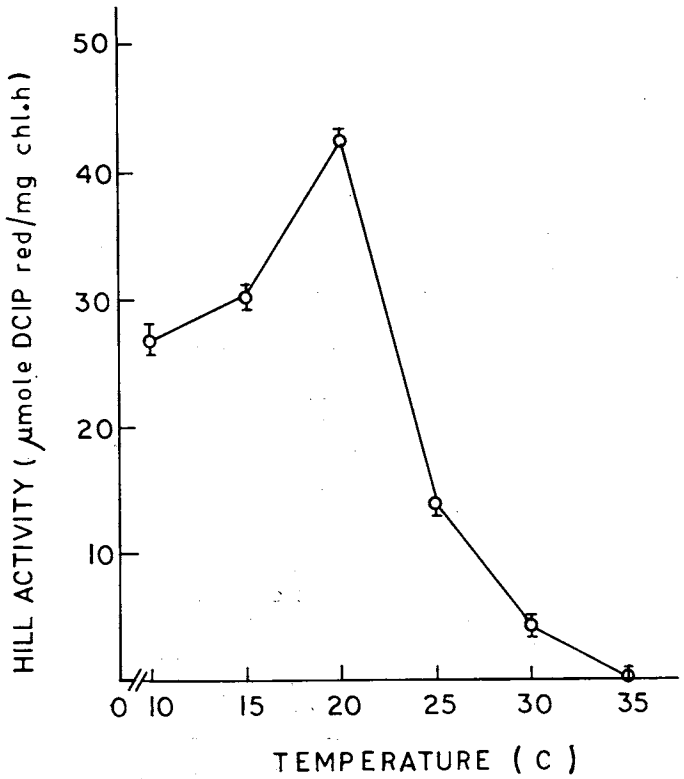

Figure 2. Effect of temperature on Hill activity (μmole DCIP red/mg chl. h) of mature leaves of sago pondweed at pH 7.5 and light intensity 60 $\mu E/m^2 \cdot sec$. The vertical bars indicate the standard errors of the means. Each point is the mean of six replications.

low temperature tolerance and high temperature inhibition of the Hill activity. This was further supported by the data that sago pondweed had a higher rate of net photosynthesis compared with hydrilla and vallisneria during the winter months (11).

Figure 3 depicts the effects of pH on the Hill activity of sago pondweed chloroplasts. Maximum Hill activity was only observed at pH 7.5 while pH levels below and above this value markedly reduced Hill activity. This clearly indicates that Hill activity is dependent upon the pH of the medium. This fits in well with the findings of a number of workers (18, 19, 22) that photosynthetic rates of several submersed macrophytes were also pH dependent. Since the Hill activity, a component of photosynthesis, was also found to be pH-dependent it might regulate the photosynthetic rate of submersed aquatic plants which encounter diurnal fluctuations in pH of their growing environment (19).

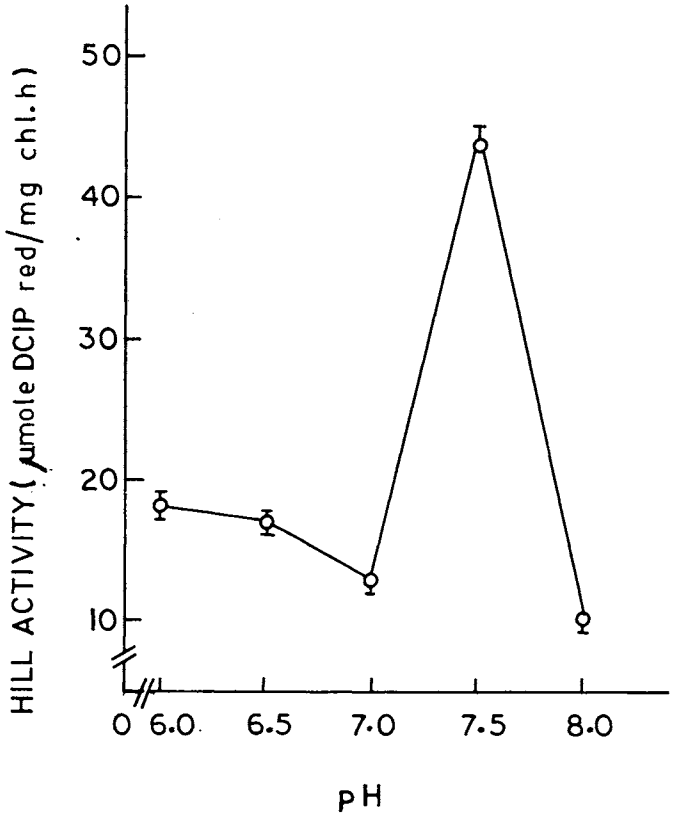

Figure 3. Effect of pH on Hill activity (μmole DCIP red/mg chl. h) of mature leaves of sago pondweed at light intensity 60 μE/m²•sec and temperature 20 C. The vertical bars indicate the standard errors of the means. Each point is the mean of six replications.

It is a general practice to use Mg^{2+} during the isolation of chloroplasts and measurement of Hill activity. It was, therefore, thought necessary to determine the effect of Mg^{2+} on Hill activity of isolated chloroplasts of sago pondweed. Figure 4 shows that the addition of Mg^{2+} ions to the assay mixture significantly enhanced the Hill activity compared with that without Mg^{2+}. Our results further reveal that the addition of Mg^{2+} in both the isolation medium as well as in the assay mixture induced greater Hill activity by chloroplasts compared with the addition of this cation in either the isolation or the reaction mixture alone. The above effect of Mg^{2+} on the Hill activity of isolated chloroplasts was also examined in spinach (Table 1) and similar effects were observed. It was clear from the results that the Hill activity was several-fold higher in spinach compared to sago pondweed and much higher light intensity (240 μE/m²•sec) was also required to get an optimum Hill activity in the former. The significantly much higher rate of Hill activity in spinach might be correlated with the observations that land plants show much higher rates of photosynthetic activities compared to submersed aquatic plants (2, 3, 16).

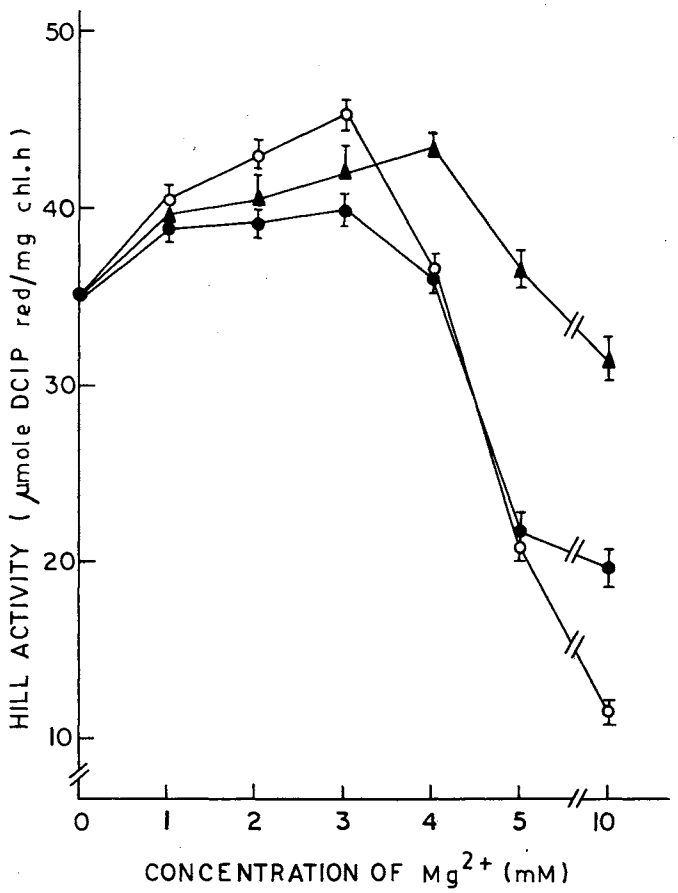

Figure 4. Effect of Mg^{2+} on Hill activity (μmole DCIP red/mg chl. h) of mature leaves of sago pondweed at pH 7.5, temperature 20 C and light intensity 60 μE/m²•sec. (●——●) Isolation without Mg^{2+} and reaction mixture with Mg^{2+}, (▲——▲) isolation with Mg^{2+} and reaction mixture without Mg^{2+}, and (O——O) isolation with Mg^{2+} and reaction mixture with Mg^{2+}. The origin indicates the Hill activity without any Mg^{2+} (control). The vertical bars indicate the standard errors of the means. Each point is the mean of six replications.

Available reports show that the reaction time for assaying the Hill activity by photoreduction of DCIP was different for different species (12, 15). It was, therefore, deemed necessary to ascertain the correct incubation time for measurement of Hill activity by isolated chloroplasts of sago pondweed. Results show that the optimum Hill activity was obtained when the incubation time is restricted to 10 min. With further increase of incubation time, the rate of photoreduction of DCIP slowed, and activity persisted only up to 90 min (Figure 5).

TABLE 1. HILL ACTIVITY (μMOLE DCIP RED/MG CHL. H) IN A SUBMERSED AQUATIC (SAGO PONDWEED) AND A TERRESTRIAL (SPINACH) ANGIOSPERM.[a]

Treatment	Hill activity	
	Sago pondweed	Spinach
Without Mg^{2+} (control)	35.0 a	203.5 a
Without Mg^{2+} in isolation and with Mg^{2+} in reaction mixture	39.5 b	225.2 b
With Mg^{2+} in isolation and without Mg^{2+} in reaction mixture	41.7 c	232.2 c
With Mg^{2+} in both isolation and reaction mixture	45.4 d	248.4 d

[a] Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of six replications.

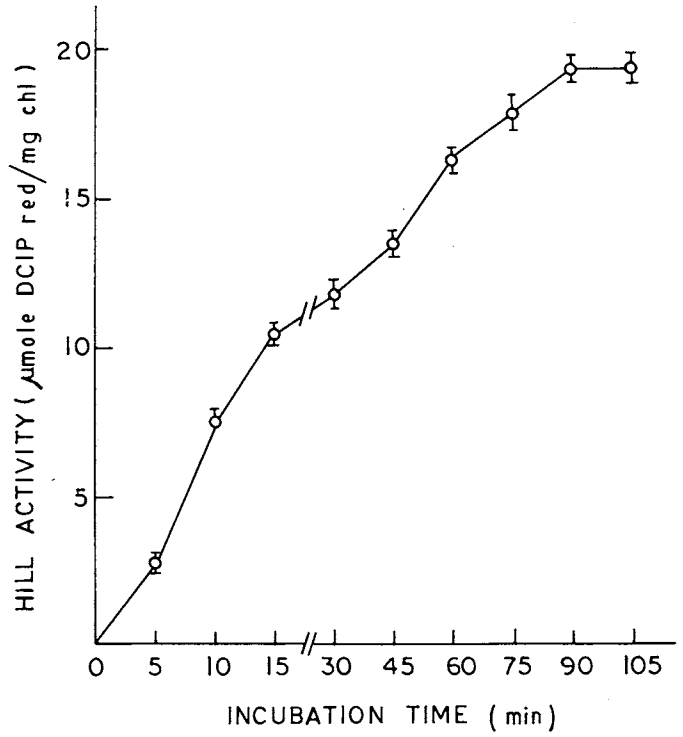

Figure 5. Effect of incubation time on Hill activity (μmole DCIP red/mg chl) of mature leaves of sago pondweed at pH 7.5, temperature 20 C and light intensity 60 $\mu E/m^2$•sec. The vehtical bars indicate the standard errors of the means. Each point is the mean of six replications.

The Hill activity under optimum assay conditions determined in previous experiments was then studied in chloroplasts isolated from three different leaf age groups of sago pondweed and spinach (Table 2). The Hill activity was highest in young leaves of sago pondweed followed by mature and old leaves. The same trend in Hill activity was also noted in case of spinach leaves of different age groups. The Hill activity of chloroplasts in isolated mature leaves of sago pondweed and spinach, induced to ageing by incubating in water in the dark for different durations, showed a declining trend with increasing incubation time and the activity became zero after 9 days of incubation (Table 3). A metabolic decline and a deterioration of isolated chloroplasts during ageing and senescence in higher plants are reported (6). The identical behaviour of the Hill activity in case of both sago pondweed and spinach suggests that the submersed aquatic plants probably behave in the same way as is generally observed in terrestrial plants during ageing, natural or induced. The photosynthetic, photorespiratory and respiratory behaviour of vallisneria during ageing as reported by Jana and Choudhuri (11) also substantiated the present proposition concerning the Hill activity of sago pondweed.

TABLE 2. EFFECT OF LEAF AGE ON HILL ACTIVITY (μMOLE DCIP RED/MG CHL. H) IN SAGO PONDWEED AND SPINACH AT PH 7.5, TEMPERATURE 20 C, LIGHT INTENSITY 60 $\mu E/M^2$•SEC (FOR SAGO PONDWEED) AND 240 $\mu E/M^2$•SEC (FOR SPINACH).[a]

Stage of leaf	Hill activity	
	Sago pondweed	Spinach
Young (20 to 30 days old)	49.9 c	259.1 c
Mature (40 to 50 days old)	45.2 b	245.1 b
Old (60 to 70 days old)	32.7 a	132.6 a

[a] Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of six replications.

TABLE 3. CHANGES IN HILL ACTIVITY (μMOLE DCIP RED/MG CHL. H) DURING AGEING OF MATURE LEAVES OF SAGO PONDWEED AND SPIN(CH.[a]

Incubation time (days)	Hill activity	
	Sago pondweed	Spinach
0	43.9 d	248.3 d
3	32.2 c	165.5 c
6	13.8 b	32.6 b
9 (senescing)	0.0 a	0.0 a

[a] Values in a column followed by the same letter are not significantly different at the 5% level as determined by Duncan's Multiple Range Test. Each value is the mean of six replications.

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