Journal of Applied Horticulture, 9(1):13-16, January-June, 2007

Developmental influence of *in vitro* light quality and carbon dioxide on photochemical efficiency of PS II of strawberry leaves (*Fragaria x ananassa*)

John H. Miranda* and Richard Williams

*School of Agronomy and Horticulture Science, The University of Queensland, Gatton, Qld.4343, Australia. * Present address: Brisbane South Lifestyle Support Services, 48 Duke Street Kangaroo Point, Q 4169, Email: j.miranda@hotmail.com*

Abstract

The influence of light quality and carbon dioxide concentration on the development of photosynthetic functional structures of strawberry leaves *in vitro* was examined. We assessed the photochemical quenching parameter of chlorophyll a photochemical efficiency of photo system II (PSII) of strawberry leaves *in vitro* in a factorial set up. The main effects of light quality; averaged over CO_2 enriched, air flow and closed systems, increased the initial chlorophyll fluorescence value from 485 for yellow light developed PS II system of leaves to 1142 for white light (control) developed ones. The photochemical efficiency of PSII significantly increased from 0.64 under white light to 0.80 for yellow light developed leaves. The leaves developed under blue light were similar to that of control white light for many chlorophyll fluorescence parameters except the initial chlorophyll fluorescence level. The increase in photochemical efficiency of PSII of strawberry leaves can be attributed to lower initial fluorescence values. Under blue light the total dry weight and total chlorophyll content were increased. The possible role of high mercury peak of white light and photoinhibition during development *in vitro* is discussed.

Key words: Carbon dioxide enrichment, air flow, photo system II, photochemical efficiency of PS II (F_V/F_M), photosynthetic photon flux (PPF), photoinhibition, dry weight, chlorophyll and nitrogen.

Introduction

The leaves developed under *in vitro* conditions usually have poorly developed chloroplasts; disorganised granal structure, low chlorophyll (chl) and protein content in general (Capellades *et al.,* 1990 and Ziv and Ariel, 1995) and the leaves of *in vitro* developed strawberry plants lack photosynthetic ability (Grout, 1988). Understanding the photosynthetic potential and changes in photosynthetic efficiency of *in vitro* developed leaves, in response to *in vitro* changes in physical environmental conditions become essential to regulate the *ex vitro* adaptation conditions for quick establishment of the plants in the open. The chlorophyll fluorescence technique offers a precise and highly sensitive measure of the photochemical efficiency of PS II and the photosynthetic capacity of the leaves (Butler, 1977) by probing the internal structure of the Photosystem II (PS II) and it's functioning. Chlorophyll fluorescence measurement is rapid, extremely sensitive, and non-intrusive and can be performed on intact leaves of field plants as well as isolated chloroplast or sub chloroplast particles (Butler, 1977; Krause and Weis, 1991 and Samson *et al.,* 1999). These parameters may be good predictors of *ex vitro* performance.

The reports on the chlorophyll fluorescence parameters of *in vitro* developed leaves under conventional system are generally varied. This is mainly due to the difference in developmental conditions of the tissue culture system, like photosynthetic photon flux (PPF) (Ticha *et al.,* 1995) or photoauto/mixotrophic condition (Haisel *et al.,* 1999; Ticha *et al.,* 1998 and Pospisilova *et al.,* 1998) or the species used (Rival *et al.,* 1997 and Triques *et al.,* 1997). There are no reports on the photochemical efficiency of PS II of strawberry leaves developed in a conventional closed tissue culture system at low light intensity (< 80 μmol m^{-2} s^{-1}) or, of any plant developed under different spectral qualities and CO_2 regimes *in vitro*. We have tested the developmental influence of light spectral qualities or CO_2 concentration on the photochemical efficiency of PS II in strawberry leaves *in vitro*, while all other parameters: controllable biological, physical and chemical conditions were constant.

Materials and methods

Plant, media and growth conditions: Strawberry cultivar 'Red Joy' plantlets of uniform size, 2-2.5cm high, ≈ 0.105 g weight, with 2-3 leaves and without roots were taken from 6-8 weeks old virus free whole plant culture developed at a rate of one plantlet per 250 mL container in 30 mL of half strength MS solid media (Murashige and Skoog, 1962) supplemented with 2 μM of BAP (pH 5.6). These cultures were maintained under a PPF of 65 ± 5.5 μmol m^{-2} s^{-1} for a 16 h photoperiod from 08:00 h to 24:00 h and at a culture growth room temperature of 26 ± 1.5°C. Strawberry plantlets were grown in 250 mL glass containers with transparent polypropylene screw lids, which were fitted with two rubber septums (Shimadzu, Japan) to suit CO_2 enrichment and air systems**.**

Light spectral qualities: The emission spectra's of light spectral qualities (Phillips, Holland) used was directly measured using a computerised spectrophotometer (Ocean Optics S2000, LAS TEK, S. Australia). A beam of light, 2 cm from the light globe which was at 1/3rd distance from the distal end of the tube light was quantified (Table 1). In addition to the respective major peaks, a mercury peak between 545 – 546 nm for white or blue or yellow

or red lights with an intensity of 1100 or 1000 or 500 or 35 units (arbitrary), respectively were also observed.

Table 1. Light quality, spectral band width and major peaks of light source used

Light spectral quality	Spectral bandwidth (nm)	Major peak wavelength (nm)
White	461 - 650	438 - 440
Blue	423 - 476	435 - 436
Yellow	537 - 637	578 - 579
Red	646 - 680	658 - 662

CO_2 enrichment and air flow systems: The plantlets were enriched with CO_2 or air as per the experimental design using a gas flow system developed at the University of Queensland, Gatton campus. This system consisted of: 1) compressed CO_2 (1000 ± 5ppm) and air cylinders with respective pressure gauge, G sized 15.0 MPa, BOC Australia, 2) a cigweld gas flow meter, Comweld Groups Pty Ltd, Australia, 3) solenoid time dosing control, 4) manifolds with ten outlets for each, 5) sterile Terumo needles and Millipore filter-Millex FG 0.2 µm, and 6) transparent culture container lids with 1 or 2 rubber septums - Shimadzu Japan. These cylinders were connected with upstream (0-30000 Kpa) and down stream (0-1500 Kpa) regulators. The down stream was connected to the flow meter, which was marked from 0-15 L min^{-1} with 30 equal divisions (set for 200 Kpa). The outlet of the flow meter was connected to a solenoid time dosing control system and the flow rate was at 1 L min^{-1}. The flow rate was standardised after measuring the changes in CO_2 *in vitro* using an infra red gas analyser, prior to the experiment considering the photosynthetic rate and CO_2 accumulation due to dark respiration. The temperature and PPF inside each container measured every half an hour for a week was used as covariates of the developmental characters.

Three plants were selected from each treatment and container with the plants were covered with aluminium foil and transferred to a laminar flow for recording fluorescence parameters. From each plant two to three matured leaves were selected for measuring chlorophyll fluorescence values of *in vitro* developed leaves during the last week (fifth) of the experimentation. Adjusted mean values for number of leaves $plant^{-1}$ were used for statistical analysis. Observations on the following developmental parameters were assessed at end of five weeks. Data recorded were analysed using Generalised Linear Model of Statistical Analysis System. The whole experiment was repeated at a later date to confirm the results of the first.

Fluorescence measurements: Chlorophyll fluorescence parameters were measured by plant efficiency analyser (Hansatech Instruments Ltd., UK) with Firmware Version PO2.001, Analyser Version PO2.01 and Summary Version P2.01. The excitation light intensity was set at saturation pulse mode (3000 µmol $m^{-2}s^{-1}$) for all observations. This high light intensity was chosen to get additional information on the potential photosynthetic ability of these leaves for direct *ex vitro* planting without going through an acclimatisation procedure as in normal tissue culture plants. The intact plants with the media were deflasked and placed in a sterile Petri dish under a laminar flow. The selected intact leaf blades were dark adapted for 15 min. and the illumination was activated and parameters F_0, F_M, F_V and time were recorded, where: F_0 is the initial level of chlorophyll fluorescence, F_M is the maximum level of chlorophyll fluorescence, F_V is the variable component of fluorescence, Time is the duration for fluorescence rise in ms and F_V/F_M (calculated) is the photochemical efficiency of PS II (Butler, 1977).

Total dry weight, total chlorophyll and nitrogen content: The plants were dried in a hot air oven at 65°C for three days and the weight at ambient temperature was recorded as the total dry weight. Total chlorophyll content was determined from whole plant extracts (Porra *et al.,* 1989). Total nitrogen content of whole plant was determined by combustion analyser, LECO CNS 2000, at a temperature of 1100°C calibrated with EDTA.

The light qualities; white, blue, yellow and red, in combination with three regimes of CO_2 treatments; i) CO_2 enriched (1000 ppm) system, ii) air flow system which was similar to the CO_2 enriched system except for CO_2, compressed air was used, and iii) a closed system which was the standard tissue culture system. The details of spectral qualities of the light source are shown in Table 1. A 4 x 3 factorial set up in a completely randomised design with 5 replications for each treatment was used. The temperature and PPF measured every half an hour inside each experimental unit (container) over a period of week prior to the experiment were used as covariates of fluorescence parameters. The general factorial model to describe the dependent variable which involved terms for each factor and also terms for possible interactions between the factors was chosen.

Results

The light quality used had major impact on the chlorophyll fluorescence parameters; F_0, time for fluorescence rise and F_V/F_M of leaves of the strawberry plants developed *in vitro* ($P<0.05$, Figs. 1, 4 and 5). Neither CO_2 regimes nor the interaction effect of Light x CO_2 had any significant influence on development of PS II.

Fluorescence parameters- F_0, F_M, F_V and time: Blue, yellow and red lights significantly reduced the mean F_0 value, when compared to the control, white light ($P<0.05$, Fig. 1). The lowest F_0 recorded was under yellow light. The F_M value (Fig. 2) of leaves developed under yellow light and F_V values (Fig. 3) of white light tend to be the lowest ($P>0.05$). The leaves developed under white and blue light recorded less time to reach fluorescence maximum (F_M), whereas those developed under yellow and red light recorded more time (Fig. 4).

Photochemical efficiency of PS II (F_V/ F_M): Yellow and red lights increased the mean F_V/F_M ratio of leaves when compared to those leaves developed under white and blue light ($P<0.05$, Fig. 5). Leaves developed under the white light, (control) recorded lowest photochemical efficiency of PS II. The mean F_V/ F_M ratio of leaves developed under red light were statistically higher than that of yellow light (Fig. 5).

Total dry weight, total chlorophyll and nitrogen content: Blue lights significantly increased the total dry weight and nitrogen contents of plants developed *in vitro* compared to the rest (Figs. 6 and 8). The total chlorophyll content was highest in blue and yellow light developed plants (Fig. 7).

Relationship between wavelength and chlorophyll a fluorescence parameters: The development of PS II of strawberry leaves was favoured at longer wavelengths than

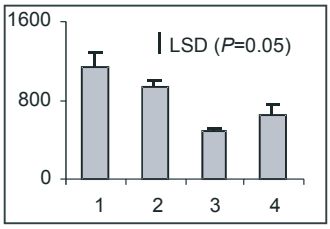

Fig. 1

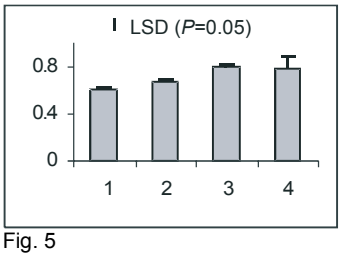

Fig. 5

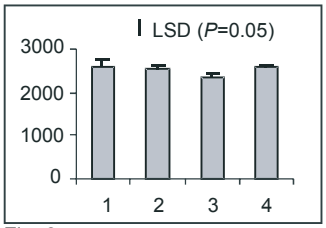

Fig. 2

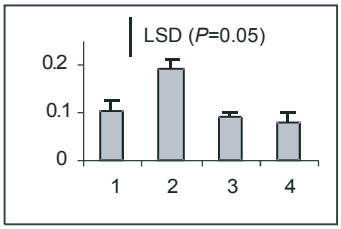

Fig. 6

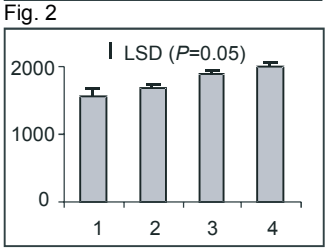

Fig. 3

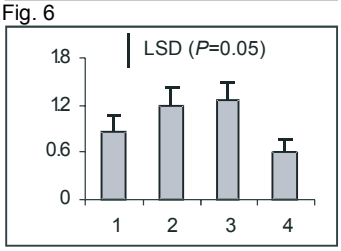

Fig. 7

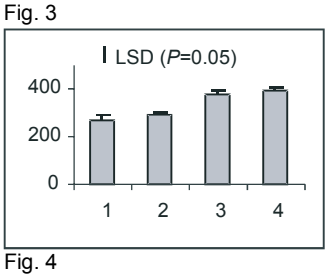

Fig. 4

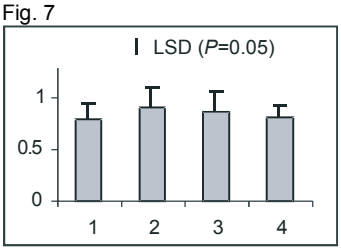

Fig. 8

Fig. 1-8. The X axis for all Figs. (1-8) represent the spectral colour of light under which the strawberry plants developed *in vitro* where; 1= white light (461-650nm), 2 = blue light (423-476nm), 3 = yellow light (537-637nm) and 4 = red light (646-680nm). In Y axes of Figs. 1-5 are chlorophyll fluorescence parameters of chl a of strawberry leaves, where Fig. 1 for mean initial chlorophyll fluorescence (F_0), Fig. 2 for mean maximum chlorophyll fluorescence (F_M), Fig. 3 for mean fluorescence variable (F_V), Fig. 4 for time for fluorescence rise from F_0 to F_M (T) and Fig. 5 for photosynthetic efficiency of PS II of Chl a (F_V/F_M), Fig. 6 for mean total dry weight (g), Fig. 7 for mean total chlorophyll content (mg g^{-1}) and Fig. 8 for mean total nitrogen content (% of total dry weight). Error bars on each mean where n=27 for each fluorescence parameter and n = 9 for all other parameters. Standard error was too small to be visible on some bars. The independent bars are least significant difference (LSD) at P =0.05.

shorter wavelengths (r = 0.875). There was a better correlation between wavelength and time for fluorescence rise (r = 0.981). There was a highly significant association between spectral peak mean wavelength and F_V (r = 0.960, $P < 0.01$).

Discussion

The light spectral qualities significantly influenced the leaf development *in vitro* whereas CO_2 regimes did not. The difference in F_0 and other parameters across lights can be due to the differences in the spectral distribution of the lights used. Though *in vitro* developed strawberry leaves lack photosynthetic ability (Grout, 1988), this study reveals that the *in vitro* developed leaves have high photochemical efficiency of PS II.

The high F_0 values and short time for fluorescence rise of chl a in leaves developed under white light can be due to the partially oxidised reaction centre or not fully active or not fully opened state instead of being fully oxidised (Figs. 5, 1 and 4). This may be a sign of photoinhibition or photodamage (Bjorkman, 1987; Krause, 1988), which occurs when the rate of degeneration of D1 protein exceeds its regeneration in the reaction centre of PS II due to unused absorbed light energy causing formation of reactive oxygen species (ROS) which fragments the D1 protein (Powels, 1984; Ohad e*t al.,* 1984; Barber, 1992; Aro *et al.,* 1993; Asada, 1999; Nikitishen e*t al.,* 2002 and Lupinkova e*t al.,* 2004). The significantly high F_0 values of white light irradiated leaves when compared to yellow or red light developed leaves may be due to the very high mercury (emission) peaks 546 – 547 nm of the spectral distribution of the light used (Fig. 1). Though the PPF was adjusted to uniformity among light qualities used, the intensity of specific peak wavelengths within the PPF was not measurable. In addition, as the CO_2 concentration reduces drastically within first four hours of light in a closed tissue culture system, as observed in this experiment and as reported previously (Falque e*t al.,* 1991; Kubota and Kozai, 1992) at CO_2 compensation point photosynthesis leads to oxygenation of ribulose 1,5- biphospate by RubisCO (Powles, 1984; Heldt, 1997) and photoinhibition or damage is possible.

The highest total dry weight, chlorophyll and nitrogen content of plants developed under blue light, irrespective of low photochemical efficiency of PS II, may be attributed to the very higher rate of photochemical (kp) reaction under blue light compared to other competing reactions in the PS II (Figs. 6-8). In the PS II photochemical reaction, $\phi P_o = k_{P\,/}\,(k_F + k_D + k_T + k_p) = (\phi F_M - \phi F_0) / \phi F_M = F_V / F_M$, where ϕP_o = Potential yield of photochemical reaction, (k_p) = the photochemical reaction, (k_F) = rate constant of pigment fluorescence, (k_D) = thermal deactivation and (k_T) = excitation energy transfer to non fluorescent pigments, are the most important competing reactions (Krause and Weis, 1991). It is known that the photosynthetic reaction demands high photon yield, and $k_P >> k_F + k_D + k_T$. When the primary quinone-type acceptor (Q_A) of PS II is oxidised (when the reaction centre is opened) the probability of fluorescence emission is low (F_0), whereas when Q_A is reduced (when the reaction centre is closed) the probability of fluorescence emission is high (FM) (Schreiber *et al.*, 1998).

In photosynthesis, the higher rate of photochemical work or the electron transfer rate or the heat released (k_D) during the transfer from second to first singlet state (Heldt, 1997) in the excitation states of chl a had a scattering effect on chlorophyll molecules than an aggregation effect during development. Thus the leaves developed under blue light with low photochemical efficiency of PS II was not a true photoinhibition and it did not have the adverse effects of chronic photoinhibition on growth, whereas leaves developed under white light had the lowest photochemical efficiency of PS II, which was due to the highest F_0 value subsequently had low dry weight and low chlorophyll and nitrogen content (Figs. 5, 1, 6 and 7). Large increase in F_0 can be due to loss of functional continuity between photon harvest and energy processing in PS II (Schreiber *et al.,* 1998) or the rate of regeneration of D1 protein was in a much slower phase than the photon harvest.

Even though photochemical efficiency of PS II was highest under

yellow and red light developed leaves, non fluorescent parameters indicated that photochemical efficiency of PS II did not have a direct effect on growth like in the blue light developed leaves. The lowest chlorophyll content under red light may be due to the root-perceived photomorphogenic inhibition of shoot greening (Tripathy and Brown, 1995) as the root system in this experiment was exposed to red light through the media (Fig. 7).

Photoinhibition under white light was caused by excess unused radiation energy from the mercury peak because the CO2 levels get depleted in a closed system within few hours of light. The highest variability of F_0 and other florescence parameters of white light developed leaves could be attributed to the influence of the mercury peak during development and warrants further investigation and due consideration may be given to an appropriate light quality to optimise in vitro plant/tissue growth.

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