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Regular paper

The regulation of component processes of photosynthesis in transgenic tobacco with decreased phosphoribulokinase activity

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Abstract

Tobacco plants (*Nicotiana tabacum* L.) transformed with an inverted cDNA encoding ribulose 5-phosphate kinase (phosphoribulokinase,PRK; EC 2.7.1.19) were employed to study the *in vivo* relationship between photosynthetic electron transport and the partitioning of electron transport products to major carbon metabolism sinks under conditions of elevated ATP concentrations and limited ribulose 1,5-bisphosphate (RuBP) regeneration. Simultaneous measurements of room temperature chlorophyll fluorescence and CO_2 gas exchange were conducted on intact leaves. Under ambient CO_2 concentrations and light intensities above those at which the plants were grown, transformants with only 5% of PRK activity showed 'down-regulation' of PS II activity and electron transport in response to a decrease in net carbon assimilation when compared to wild-type. This was manifested as a decline in the efficiency of PS II electron transport ($\Phi_{PS\,II}$), an increase in dissipation of excess absorbed light in the antennae of PS II and a decline in : total linear electron transport (J_1), electron transport dedicated to carbon assimilation (J_A) and electron transport allocated to photorespiration (J_L). The transformants showed no alteration in the Rubisco specificity factor measured *in vitro* and calculated *in vivo* but had a relatively smaller ratio of RuBP oxygenation to carboxylation rates (v_o/v_c), due to a higher CO_2 concentration at the carboxylation site (C_c). The relationship between $\Phi_{PS\,II}$ and Φ_{CO_2} was similar in transformants and wild-type under photorespiratory conditions demonstrating no change in the intrinsic relationship between PS II function and carbon assimilation, however, a novel result of this study is that this similar relationship occurred at different values of quantum flux, J_1, J_A, J_L and v_o/v_c in the transformant. For both wild-type and transformants, an assessment was made of the possible presence of a third major sink for electron transport products, beside RuBP oxygenation and carboxylation, the data provided no evidence for such a sink.

Abbreviations: C_c – CO_2 concentration at the site of carboxylation; C_i – intercellular CO_2 concentration; g_m – mesophyll conductance to CO_2; J_1 – total linear electron flow; J_A – linear electron flow allocated to CO_2 assimilation; J_c – linear electron flow supporting carbon reduction and oxidation cycles; J_L – linear electron flow allocated to photorespiration (RuBP oxygenation and fixation of released photorespiratory CO_2); PRK – phosphoribulokinase; q_P, q_N – coefficients for photochemical and non-photochemical quenching of fluorescence respectively; Rubisco – ribulose 1,5-bisphosphate carboxylase-oxygenase; S – Rubisco specificity to CO_2/O_2; v_c, v_o – rates of RuBP carboxylation and RuBP oxygenation, respectively; Φ_{CO_2} – relative quantum yield of CO_2 assimilation; Φ_C – maximum Φ_{CO_2} under non-photorespiratory conditions; Φ_{exc} – the efficiency of excitation capture by open PS II centres; $\Phi_{PS\,II}$ – relative quantum yield of PS II electron transport

Introduction

The operation of photosynthesis is often conceptualised in terms of factors limiting its component processes under defined conditions. At extremes of light input, the capacity of the light reactions to provide ATP and NADPH is limiting at low light while at high light the activity of the carbon reduction cycle is usually the limiting factor to overall photosynthetic rate. In natural ecosystems, fluctuations exist in light and temperature and the supply of water and nutrients varies yet leaf photosynthesis regulates its component processes to prevent major limitations to either the light reactions or carbon metabolism such that a balance exists between the rate of production of electron transport products by the light reactions and the rate of their utilisation by carbon metabolism. Studies of the consequences of decreased capacity for carbon assimilation upon the relationship between electron transport and carbon metabolism and the partitioning of electron transport products to RuBP carboxylation and oxygenation have mainly employed leaf material subjected to short term changes in gases, irradiance or to stress conditions (Peterson 1989; Foyer et al. 1990; Harbinson et al. 1990; Cornic and Briantais 1991; Valentini et al. 1995). Production of transformed plants with specific alterations to enzymes of carbon metabolism offer a unique opportunity to study the consequences of long term modifications in carbon assimilation upon the operation and regulation of photosynthesis (Stitt and Schulz 1994). A recent study of the effects of a reduction in phophoribulokinase activity on the operation of the carbon reduction cycle, in tobacco plants transformed with antisense PRK constructs, has shown that adjustments are made in metabolite concentrations to accommodate the loss in enzyme activity (Paul et al. 1995). Transformed plants with PRK below 15% of wild-type concentration showed over two-fold higher concentrations of precursors to RuBP regeneration (ribulose 5-phosphate, ribose 5-phosphate, ATP and fructose 6-phosphate) and over two-fold lower concentrations of products of PRK reaction (ribulose 1,5-bisphosphate, 3-phosphoglyceric acid and ADP). Such transformants with altered PRK activities are examined in this study to determine the consequences of the dramatically lowered RuBP concentration at high ATP concentrations on: (1) the partitioning of electron transport products between Rubisco carboxylation and oxygenation, (2) the relationship between electron transport and carbon metabolism and (3) the consequences to Rubisco specificity factor estimated *in vivo* and measured *in vitro*.

Studies combining chlorophyll fluorescence and gas exchange techniques for the calculation of the partitioning of electron transport products between the carboxylation and oxygenation functions of Rubisco have yielded information on the role of photorespiration under water stress (Cornic and Briantais 1991; Brestic et al. 1995; Valentini et al. 1995), allowed the calculation of v_o/v_c in wheat leaves developed under elevated CO_2, (Habash et al. 1995) and provided an estimate of Rubisco specificity factor and mesophyll conductance *in vivo* (Ghashghaie and Cornic 1994; Epron et al. 1995). The calculations involved in these studies assume that alternative routes for linear electron transport, such as the Mehler reaction, are mimimal. This supposition has been supported by the agreement of calculated values for Rubisco specificity factor and mesophyll conductance obtained using the combined fluorescence and gas exchange technique with values obtained using fundamentally different methodology (Peterson 1989; Cornic and Brianitais 1991; Ghashghaie and Cornic 1994). It has recently been argued that the Mehler reaction could potentially consume a large proportion of linear electron transport in leaves (Osmond and Grace 1995). An assessment is made in this study of possible alternative sinks for electron transport products, in addition to RuBP carboxylation and oxygenation, in wild-type and transformed tobacco leaves using combined fluorescence and gas exchange techniques.

Materials and methods

Growth conditions

Two types of tobacco (*Nicotiana tabacum* L.) plants were used in this study. Tobacco was transformed with *Agrobacterium tumefaciens* containing either the binary vector pBIN19 producing plants with wild-type concentrations of PRK (referred to as wild-type) or a pBIN19 with antisense PRK construct producing plants with 5% of wild-type PRK activity (referred to as transformant) (Paul et al. 1995). Seed was sown and germinated at 25 °C on filter paper in Petri dishes. The seedlings were transferred to a peat-based soil (Eff compost; Croxdens, UK) and grown in controlled-environment conditions of 25 °C day/night temperature, 70% relative humidity and 280–340 μmol m^{-2} s^{-1} quantum flux measured at the uppermost leaf.

Plants were provided with nutrient solution twice a week (Vitafeed 412; Vitax Ltd, Skelmersdale, UK). Mature fully expanded leaves from 4–6-week-old plants were used in the experiments.

Gas-exchange measurements

Gas-exchange was measured on attached leaves using a multichamber open-circuit system the details of which are described in Habash et al. (1995). Light-response curves, at constant chamber CO_2 concentration and 25 °C, were determined at steady state photosynthesis allowing an average of 40 minute equilibration at each quantum flux; quantum flux was measured with a quantum sensor (LI-189; LI-COR-Inc, Lincoln NE, USA). The response of net carbon assimilation to intercellular CO_2 concentration, C_i, was determined after one hour equilibration at each gas concentration. Leaf temperature was calculated from the energy budget of the leaf (Ehleringer 1989). Calculations of assimilation and transpiration rates, stomatal conductance to water vapour and intercellular CO_2 concentration were based on the equations of von Caemmerer and Farquhar (1981). The relative quantum yield of CO_2 fixation, Φ_{CO_2}, was calculated as $(A + R_{dark})$/incident photon flux.

Fluorescence measurements

Room temperature PS II chlorophyll fluorescence was measured at steady-state photosynthesis on intact attached leaves in a specially designed gas-exchange chamber allowing measurement of gas exchange and fluorescence simultaneously as described in Habash et al. (1995). The protocol for the measurements was similar to that presented in Genty et al. (1989) and the fluorescence terminology is that recommended by van Kooten and Snel (1990). The following fluorescence parameters were measured: maximal fluorescence induced by a saturating pulse at steady-state photosynthesis (F_m'), maximal fluorescence induced by a saturating pulse after one hour dark adaptation (F_m), steady-state fluorescence under continuous illumination (F_s), minimal level of fluorescence at steady state photosynthesis (F_0') and minimal level of fluorescence after one hour of dark adaptation (F_0). The relative quantum yield of PS II electron transport ($\Phi_{PS\,II}$) is defined as $(F_m' - F_s)/F_m'$, the efficiency of excitation capture by open PS II centres (Φ_{exc}) as $(F_m' - F_0')/F_m'$ (Genty et al. 1989), photochemical quenching of fluorescence (q_P) as $(F_m' - F_s)/F_m' - F_0')$ and non-photochemical quenching of fluorescence (q_N) as $1-(F_m' - F_0')/F_m\text{-}F_0$) (Schreiber et al. 1986).

Calculation of electron-transport rate in leaves

The rate of total linear electron transport, J_1, in leaves under photorespiratory conditions was calculated from $J_1 = (\Phi_C)$ (incident quantum flux) (4). Φ_C was the maximum Φ_{CO_2} obtained from a 'calibration line' constructed from the linear relationship between $\Phi_{PS\,II}$ and Φ_C measured under non-photorespiratory conditions (2100 μmol mol^{-1} CO_2 and 20 mmol mol^{-1} O_2) for each set of plants, wild-type and transformants. Linear regression was performed and the equation $\Phi_C = a(\Phi_{PS\,II}) - b$ for transformant and wild-type was used in the calculation of linear electron flow J_1. The allocation of electron-transport products to CO_2 fixation ($J_A = 4(A + R_d)$) and photorespiration ($J_L = J_1 - J_A$) was as detailed in Habash et al. (1995) following the method developed by Ghashghaie and Cornic (1994) where RuBP carboxylation and oxygenation were the only sinks for linear electron transport ($J_1 = J_A + J_L$). The rate of Rubisco caboxylation, $v_c = (A + R_d + 0.5\,v_o)$ and the rate of Rubisco oxygenation $v_o = J_L/6$ were calculated with R_d, the rate of respiration in the light other than photorespiration, determined from A/C_i curves according to the method described in Brooks and Farquhar (1985). R_d determined thus was set at $0.5(R_{dark})$ where R_{dark} was the rate of respiration at steady state in the dark. Total electron transport supporting RuBP carboxylation and oxygenation (J_c) was calculated from $J_c = (A + R_d)4((C_i - A/g_m) + \Gamma^*)/(C_i - A/g_m) - \Gamma^*)$ according to von Caemmerer and Farquhar (1981). g_m, the mesophyll conductance to CO_2, of 0.3 μmol m^{-2} s^{-1} bar^{-1} was taken from von Caemmerer et al. (1994) as a representative value for tobacco. Γ^*, the concentration of CO_2 at which the rate of carboxylation equals the rate of photorespiratory CO_2 evolution, of 38 μmol mol^{-1} CO_2 was determined according to Brooks and Farquhar (1985).

Calculation of mesophyll conductance (g_m), CO_2 concentration at the carboxylation site (C_c) and CO_2/O_2 specificity of Rubisco (S) in leaves

Mesophyll conductance to CO_2, g_m, and the CO_2 concentration at the carboxylation site, C_c, was calculated from $g_m = A/\{C_i - [(\Gamma^*(J_1 + 8(A + R_d))/(J_1 - 4(A + R_d))]\}$ according to Harley et al. (1992). Total linear electron flow J_1, A, C_i and leaf temperature were calculated for each quantum flux in a light-response curve.

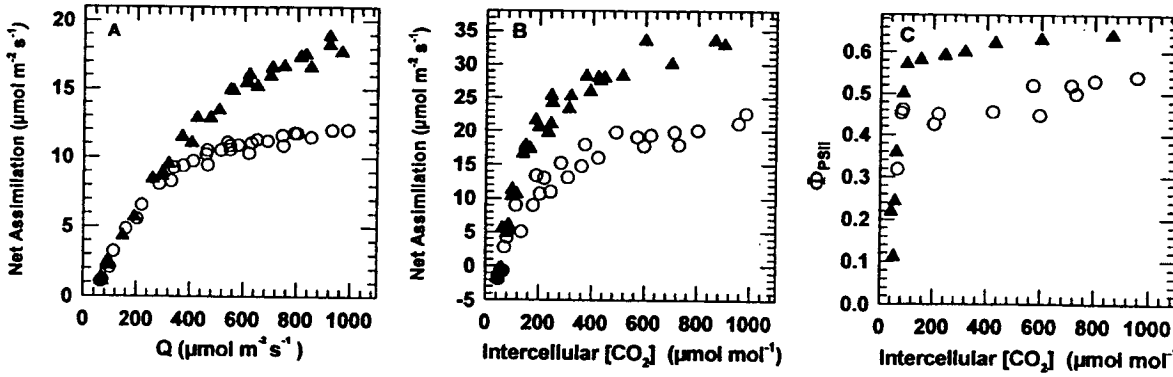

Figure 1. (A) Response of net CO_2 assimilation to incident quantum flux under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 2 mmol mol^{-1} O_2), (B) net CO_2 assimilation and (C) $\Phi_{PS\,II}$ as a function of intercellular CO_2 concentration at 900 μmol m^{-2} s^{-1} incide quantum flux and 210 mmol mol^{-1} O_2 for wild-type (▲) and transformant (○) plants. (A and B) data are from 5 and 4 different plants for ea type respectively and plot C shows representative data from one leaf.

The CO_2/O_2, specificity of Rubisco (S) was calculated in two ways: (1) from $v_c/v_o = S([CO_2]/[O_2])$ following Laing et al. (1974) with v_c and v_o calculated as above, $[CO_2] = C_c$ and $[O_2]$ the concentration of O_2 in the leaf obtained from physical tables of the solubility of O_2 in water at a given temperature, and (2) from $\Gamma^* = 0.5[O_2]/S$ according to Brooks and Farquhar (1985).

Purification of Rubisco and measurement of specificity factor

Rubisco was isolated and purified from 5-week-old transformed and wild-type tobacco plants as described in Keys and Parry (1990). The extraction buffer was specifically optimised for tobacco with the following composition: 20 mol m^{-3} HEPES, 10 mol m^{-3} $MgCl_2$, 10 mol m^{-3} $NaHCO_3$, 1 mol m^{-3} EDTA, 100 mol m^{-3} 2-mercaptoethanol, 1 mol m^{-3} phenylmethanesulphonylfluoride, 2% (w/v) bovine serum albumin, 1% (w/v) polyvinyl pyrollidone, pH 8.0. Rubisco specificity factor was determined using [1-^{14}C] ribulose 1,5-bisphosphate as substrate and measuring ^{14}C in 3-phospho-D-glycerate and 2-phosphoglycolate following separation by HPLC (Bainbridge et al. 1995).

Results

The rates of net CO_2 assimilation as a function of quantum flux in intact attached leaves of wild-type tobacco plants and plants with 5% of wild-type PRK activity are shown in Figure 1A. At quantum flux above 340 μmol m^{-2} s^{-1}, the rate of assimilation was smaller in the transformant compared to the wild-type and this was reflected in a change in the shape of the lig response curve indicating an increased limitation light above 340 μmol m^{-2} s^{-1}. The rate of assimilati was unchanged at the lower quantum flux indicati no difference in the apparent quantum yield of C assimilation in both plants which is expected sin PRK does not exert any control at the lower qua tum flux. Transformants exposed to a quantum flux 1000 μmol m^{-2} s^{-1} for one hour and transferred 350 μmol m^{-2} s^{-1} gave similar steady state assim lation rates when measured at the lower quantum flu compared to plants exposed to and measured at t lower quantum flux (data not shown) indicating th no damage to the photosynthetic apparatus occurre during the period of exposure to the higher quantu flux. The transformants had a 33% decrease in chlor phyll content per leaf area, this did not, however, lim photosynthesis, since the absorptivity of the leaf wa only 3% less than the wild-type (Paul et al. 1995). Th response of net CO_2 assimilation to a range of C concentrations showed little change at the lower C_i b was reduced in the transformant as CO_2 increased, thu reflecting the limitation imposed by RuBP regener tion (Figure 1B). The higher photosynthetic capaci in the wild-type was also demonstrated when Φ_{PS} was measured at various CO_2 concentration (Figu 1C). Further, the plot showed that for leaves from bo types, the apparent quantum yield of PS II electro transport remained relatively high for a range of C_i b started to decline sharply at C_i below 100 μmol mol^{-} This demonstrates the persistence of a substantial flu of electron transport products to photorespiration RuBP carboxylation is reduced.

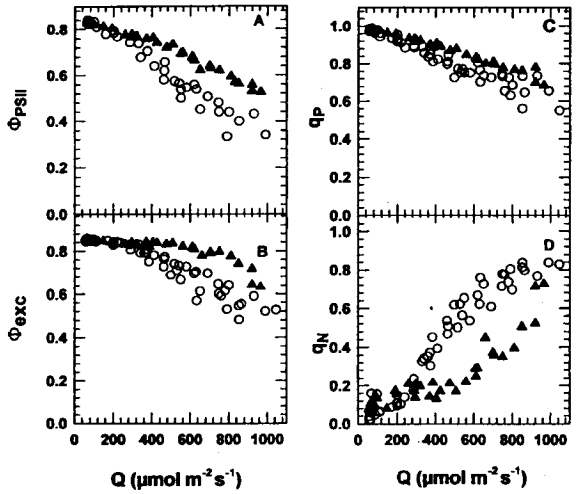

Figure 2. Fluorescence parameters as a function of incident quantum flux for wild-type (▲) and transformant plants (○): (A) The relative quantum yield of PS II electron transport, $\Phi_{PS\,II}$, (B) the photochemical efficiency of open PS II centres, Φ_{exc}, (C) the coefficient of photochemical quenching of fluorescence, q_P, and (D) the coefficient of non-photochemical quenching of fluorescence, q_N. Experiments were performed under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 210 mmol mol^{-1} O_2); data are from 5 different plants of each type.

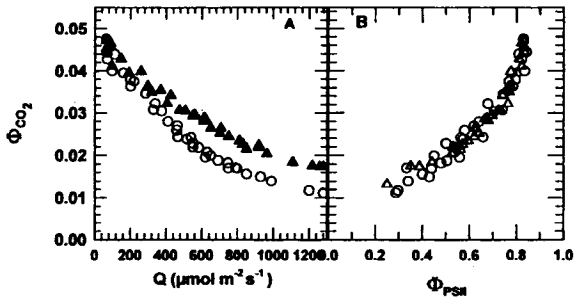

Figure 3. (A) The response of the relative quantum yield of CO_2 assimilation, Φ_{CO_2}, to incident quantum flux for wild-type (▲) and transformant (○) plants. (B) The relationship between Φ_{CO_2} and $\Phi_{PS\,II}$ for wild-type (△) and transformant (○) plants. Experiments were performed under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 210 mmol mol^{-1} O_2); data are from 5 different plants of each type.

A concomitant lowering of the relative quantum yield of PS II electron transport occurred as carbon assimilation was decreased in the transformants (Figure 2A). To understand the reason behind the reduction in $\Phi_{PS\,II}$, plots of Φ_{exc} and of q_P (the product of which yields $\Phi_{PS\,II}$ (Genty et al. 1989)) versus light are shown in Figure 2. It is clear that both the efficiency of excitation capture by open PS II centres, Φ_{exc} (Figure 2B), and the estimated population of open PS II centres, q_P (Figure 2C), are decreased in the transformant at quantum flux greater than 340 μmol m^{-2} s^{-1}. Further, the parameter q_N, which represents the overall non-photochemical quenching of fluorescence, increased in the transformant at the same quantum flux where changes in Φ_{exc} and q_P occurred (Figure 2D). Therefore, it is clear that the changes involved in the decrease in carbon assimilation through diminished expression of PRK are sensed at the level of PS II.

The decline in $\Phi_{PS\,II}$ (Figure 2A) was paralleled by a lowering in Φ_{CO_2} (Figure 3A) as a function of quantum flux under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 210 mmol mol^{-1} O_2). A plot of Φ_{CO_2} as a function of $\Phi_{PS\,II}$ under photorespiratory conditions is curvilinear and similar for both the transformant and wild-type leaves (Figure 3B). This means that the reduction in PRK concentration and thus RuBP regeneration potential in the transformant did not alter the intrinsic relationship between PS II electron transport and RuBP carboxylation. However, this correspondence in the two efficiencies occurred at different incident photon fluxes, i.e., an equal $\Phi_{PS\,II}$ of 0.65 and Φ_{CO_2} of 0.0275 was obtained at incident quantum fluxes of 400 and 600 μmol m^{-2} s^{-1} in the transformant and wild-type leaves respectively.

The partitioning of electron transport products between RuBP carboxylation and RuBP oxygenation required the construction of a calibration plot to determine the maximum relative quantum yield of CO_2 assimilation (Φ_c) for a given $\Phi_{PS\,II}$ obtained under non-photorespiratory conditions of 2100 μmol mol^{-1} CO_2 and 20 mmol mol^{-1} O_2 (Ghashghaie and Cornic 1994; Habash et al. 1995). Such a plot of Φ_c and $\Phi_{PS\,II}$ showed a linear correlation for wild-type and transformants with a slight reduction in Φ_c at a given $\Phi_{PS\,II}$ in the transformant (Figure 4A). Plots of electron flux as a function of quantum flux for wild-type and transformed plants are shown in Figure 4B–F. The partitioning of electron transport products to carboxylation and photorespiration showed a larger allocation of electron transport products to photorespiration in the wild-type (Figure 4B) compared to the transformant (Figure 4C). There was also a substantial reduction in the absolute values of J_1, (Figure 4D), J_A (Figure 4E) and J_L (Figure 4F) in the transformant compared to the wild-type. It is also interesting to note that the shapes of these plots are different with a flattening of J_1 and J_A at quantum flux above 400 μmol m^{-2} s^{-1} in the transformant. Linear electron transport satisfying the consumption of NADPH by RuBP carboxylation and oxygenation, J_c, was calculated using gas exchange

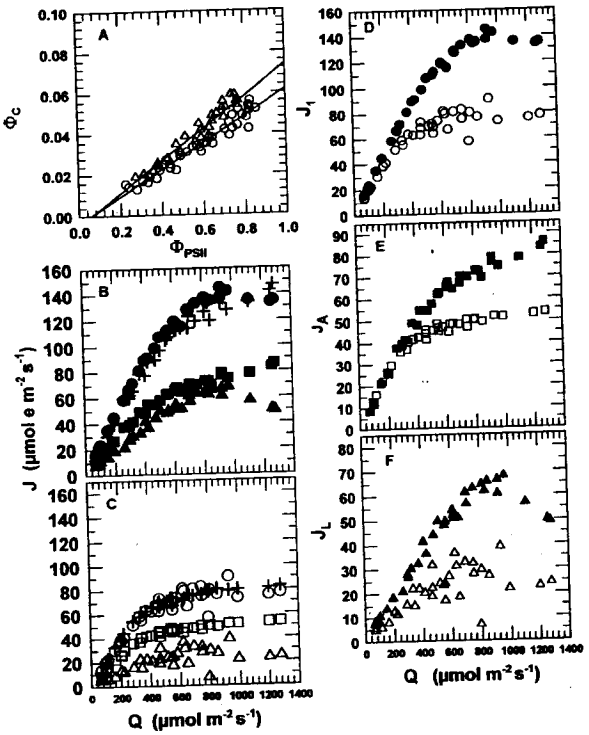

Figure 4. (A) The relationship between Φ_C and $\Phi_{PS\,II}$ under non-photorespiratory conditions (2100 μmol mol^{-1} CO_2 and 20 mmol mol^{-1} O_2) for wild-type (Δ) and transformant (○) plants; data are from 4 different plants for each type. Linear regression was performed and the equations $\Phi_C = 0.0654\ \Phi_{PS\,II} - 0.0037$ ($r^2 = 0.947$) for transformant and $\Phi_C = 0.0786\ \Phi_{PS\,II} - 0.0046$ ($r^2 = 0.853$) for wild-type were used in the calculation of electron transport. (B,C) Linear electron transport, J_1, (circle), electron transport to CO_2 assimilation, J_A, (square) and electron transport to photorespiration, J_L, (triangle) as a function of incident quantum flux for wild-type (B, closed symbols) and transformant plants (C, open symbols). Crosses represent electron transport J_c dedicated to RuBP carboxylation and oxygenation calculated from gas exchange parameters according to $J_c = (A + R_d)4((C_i - A/g_m) + 2\Gamma^*)/(C_i - A/g_m) - \Gamma^*)$. (D–F) The response of J_1 (D), J_A (E) and J_L (F) to incident quantum flux for wild-type (closed symbols) and transformant (open symbols) plants. (B–F) Experiments were performed under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 210 mmol mol^{-1} O_2); data are from 5 different plants of each type.

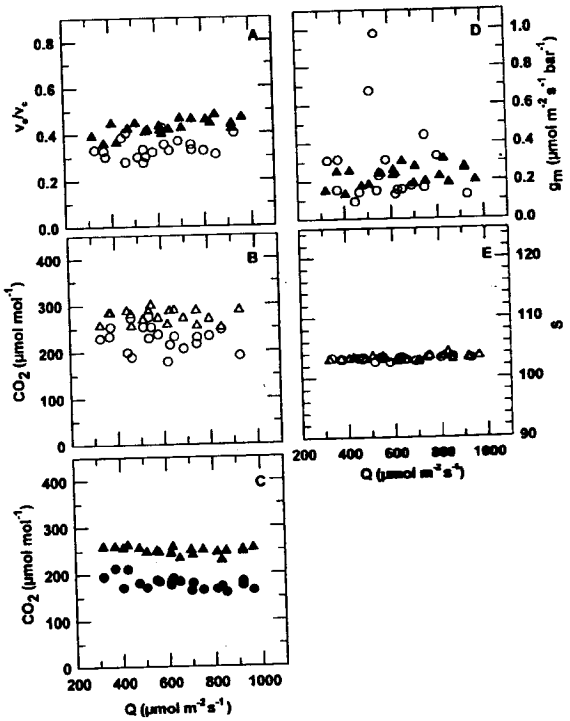

Figure 5. (A) The change in the ratio of RuBP oxygenation to carboxylation, v_o/v_c, with incident quantum flux for wild-type (▲) and transformant (○) plants. (B,C) The response of intercellular CO_2 concentration, C_i, (triangle) and CO_2 concentration at the site of carboxylation, C_c, (circle) to incident quantum flux for transformant (B) and wild-type (C) plants. (D) The response of the mesophyll conductance to CO_2, g_m, to incident quantum flux for wild-type (▲) and transformant (○) plants. (E) The response of Rubisco specificity factor, S, to incident quantum flux for wild-type (Δ) and transformant plants (○). Experiments were performed under photorespiratory conditions (350 μmol mol^{-1} CO_2 and 210 mmol mol^{-1} O_2); data are from 5 different plants of each type.

parameters according to von Caemmerer and Farquhar (1981) with g_m set at 0.3 μmol m^{-2} s^{-1} bar^{-1} (Figure 4B,C). Comparison of the measured rates of total linear electron transport, J_1, determined using simultaneous measurements of gas-exchange and fluorescence, with the calculated rates of electron transport supporting carbon assimilation and photorespiration, J_c, were very similar. The presence of a substantial alternative sink for the products of total linear electron transport would have showed larger values for J_1. The fact that this did not occur provided a demonstration that in both wild-type and transformed tobacco leaves, under the conditions examined, RuBP carboxylation and oxygenation were the two major sinks for linear electron transport.

The ratio, of v_o/v_c was smaller in the transformant than the wild-type at all quantum fluxes with mean values of about 0.33 compared to 0.45, respectively (Figure 5A). This smaller ratio in the transformant was due to a greater concentration of CO_2 at the carboxylation site, C_c, (Figure 5B) of about 55 μmol mol^{-1} at quantum fluxes higher than 400 μmol m^{-2} s^{-1} compared to wild-type (Figure 5C). The calculated mesophyll conductance to CO_2 was similar in both sets of plants with the exception of a few data points showing a large value for the transformant (Figure 5D), these reflect the close C_c to C_i values in the transformant (Figure 5B). The absolute value for mesophyll conductance calculated

from the data at high quantum flux was 0.28 μmol m^{-2} s^{-1} bar^{-1} and this compares with values of 0.3 (von Caemmerer et al. 1994) and 0.37 (Evans et al. 1994) μmol m^{-2} s^{-1} bar^{-1} obtained for wild-type tobacco using carbon isotope discrimination techniques; the slightly lowered absolute value obtained in this study could relate to different growth conditions. The specificity of Rubisco to CO_2/O_2, calculated in vivo from ($v_c/v_o = S[CO_2]/[O_2]$) using the combined fluorescence and gas exchange technique, was the same, 102, for both types of plants (Figure 5E). Further, S was also calculated according to Brooks and Farquhar (1985) using gas exchange data alone from Γ^* and $[O_2]$ ($\Gamma^* = 0.5[O_2]/S$): values of 101 and 105 for wild-type and transformants, respectively, were obtained. These calculated values *in vivo* are close to S of 97.5 recently obtained for wild-type tobacco *in vivo* using a g_m of 0.3 μmol m^{-2} s^{-1} bar^{-1} (von Caemmerer et al. 1994). Rubisco specificity factor was also measured on purified protein and means of 89 (±8) and 91 (±9) were obtained for transformant and wild-type respectively. These were slightly lower than the absolute values calculated from measurements made *in vivo* on similar leaves. Previous studies have shown that S estimated *in vivo* is usually about 10–20% higher than values measured *in vitro*; the reasons for this discrepancy are not clear and may be due to the different environment and enzyme concentrations *in vivo* (von Caemmerer et al. 1994). It is important to point out that when a value of S = 89, reported as the best measured value *in vitro* for tobacco (Parry et al. 1987), was incorporated in the calculations of S *in vivo* for both types of plants, using v_c and v_o calculated in this study, the consequence was to increase the calculated C_c, to values above the measured C_i for most data points, and in some above ambient CO_2 values (calculations not shown).

Discussion

The decrease in the net assimilation rate of CO_2 in tobacco plants with 5% of wild-type PRK activity at quantum fluxes greater than 340 μmol m^{-2} s^{-1} is due to the lower enzyme concentration which results in over two-fold drop in RuBP concentration and thus presents a major limitation to photosynthesis at quantum fluxes above those which the plants were exposed to during growth. At lower quantum flux there is no difference in net photosynthesis or PS II activity between the two type of plants since PRK is not limiting under these conditions.

The decrease in overall PS II activity and, consequently, in linear electron transport measured in this study is the primary regulatory mechanism that operates at the thylakoid level in the transformants. The increase in non-photochemical quenching of fluorescence, q_N, dissipates the extra absorbed energy, not consumed by carbon metabolism, as heat and prevents overreduction of the electron transport chain. The reduction in the efficiency of energy transduction in open PS II centres, Φ_{exc}, or the reduction in the intrinsic quantum yield of open PS II centres indicates the onset of q_N. The lowered assimilation rate was also reflected in the reduction in photochemical quenching and the estimate of the number of open or oxidised PS II centres in the transformant. This demonstrates the fine tuning of the redox state of Q_A to the rate of utilisation of electron transport products by carbon assimilation. Similar changes in PS II were obtained in tobacco with decreased amounts of Rubisco (Stitt et al. 1991). These changes at PS II have been demonstrated in studies on wild-type leaves under a range of conditions and may be described as 'down-regulation' in that they are a preventative measure to minimise damage to thylakoid components (Foyer et al. 1990).

The small difference in the relationship between $\Phi_{PS\,II}$ and Φ_{CO_2} under non-photorespiratory conditions (Figure 4A) was surprising. It is conceivable that inaccuracies in estimating R_d might be responsible for this difference. Therefore, we have altered the values of R_d up to 38% and observed that it had a minor influence (a 7% change in Φ_C) on the data only at the very high efficiency values at low quantum flux. Further, this did not account for the rest of the plot since R_d contributes less to Φ_C at high quantum flux. We also used the regression line of the wild-type for the transformants in the calculations and the consequence was to increase the calculated Rubisco specificity factor far beyond the measured values. Another explanation for the difference in the regression lines might be the existence of a significant Mehler reaction in the transformed plants; this option was not supported by the similar values obtained for J_l and J_c for both wild-type and transformants. It is worth noting that there has been a report in the literature showing differences in similar plots in mutant barley with lowered levels of glutamine synthetase and ferredoxin-dependent glutamate synthase (Hausler et al. 1994).

The relationship between $\Phi_{PS\,II}$ and Φ_{CO_2} under photorespiratory conditions is a function of the absorption of light by the leaf, the distribution of photons to PS II and PS I, the stoichiometry of electrons needed

to reduce CO_2 and alternative electron transport sinks such as photorespiration, Mehler reaction and nitrite reduction (Edwards and Baker 1954). These factors can vary between treatments such as under cold stress (Harbinson et al. 1990) and in different plants thus producing changes in the relationship between the efficiencies. This study has shown the transformant to be less efficient in photosynthesis at higher quantum flux compared to the wild-type. However, we have demonstrated that the similar relationship between $\Phi_{PS\,II}$ and Φ_{CO_2} under photorespiratory conditions occurred at different incident quantum flux and with a different balance between the component processes in photosynthesis in the transformed plants (i.e. at different J_1, J_A, J_L and v_o/v_c). Thus, for the wild-type, a Φ_{CO_2} of 0.0275 was obtained at a quantum flux of 600 μmol m^{-2} s^{-1}, $\Phi_{PS\,II}$ of 0.65, J_1, J_A, and J_L of 120, 67, 53 μmol e m^{-2} s^{-1}, respectively, and v_o/v_c of 0.42. However, the same values for Φ_{CO_2} of 0.0275 and $\Phi_{PS\,II}$ of 0.65 for the transformant were obtained at a quantum flux of 400 μmol m^{-2} s^{-1} and J_1, J_A, and J_L of 70, 45, 25 μmol e m^{-2} s^{-1}, respectively, and v_o/v_c of 0.32 (see Figures 2, 4 and 5). This novel aspect of the relationship between $\Phi_{PS\,II}$ and Φ_{CO_2} demonstrates the ability of the photosynthetic system to adjust its partial reactions at various poises to maintain a consistent relationship between the functioning of the light reactions and carbon assimilation

The lowered flux of electron transport to photorespiration and the smaller ratio of v_o/v_c in the transformant relative to the wild-type point to a decrease in the relative rate of photorespiration. The reason for the decrease in photorespiration could be due both to the higher C_c in the transformant (Figure 5B) and to the dramatically lower concentration of RuBP (Paul et al. 1995). Recent studies have also demonstrated a higher Rubisco activation state in the transformants (Paul et al. unpublished). The changes in v_o/v_c in response to fluctuating C_c reflect the uniformity of the Rubisco specificity factor. The constancy of both the specificity of Rubisco for CO_2/O_2 and the ratio v_o/v_c as a function of quantum flux at constant leaf temperature (Figures 5E and A) is expected from the known kinetic properties of Rubisco. The values of v_o/v_c obtained for both types of plants (Figure 5A) lie within the range 0.3–0.5 expected under atmospheric conditions (Laing et al. 1974). It is important to point out that a strategy of increasing the flux through photorespiration as a safety valve to dissipate excess ATP and NADPH at high quantum flux is not seen in this study for the transformed plants, but what is clearly obtained is the primary 'down-regulation' of PS II which handles the excess energy over what is needed to maintain carbon assimilation and produces ATP and NADPH at a rate proportional to their utilization.

The data obtained with the transformant and wild-type leaves demonstrate that the occurrence of a third large sink for electron transport products, such as the Mehler reaction, is unlikely under the conditions examined. It is reasonable to assume that if such a sink were present then the rate of total linear electron transport would be maintained and the partitioning of products to Mehler reaction, RuBP carboxylation and oxygenation would change. This clearly does not happen in the transformant since there is a proportional 'down-regulation' of linear electron transport, J_1, with the decrease in carbon assimilation. Furthermore, the closeness of J_1 and J_c (Figure 4B,C) demonstrates that RuBP carboxylation and oxygenation are the major sinks for total linear electron transport; a third sink would have resulted in higher J_1 than J_c. The stimulation in carbon assimilation observed upon the removal of photorespiration by reduction of $[O_2]$ and increase in $[CO_2]$ in both the transformant and wild type (compare Figure 3B and Figure 4A) and the persistence of a high $\Phi_{PS\,II}$ at low CO_2 and 210 mmol mol^{-1} O_2 (Figure 1C) also support photorespiration as the other major sink for electron transport products in tobacco leaves. These considerations, plus the closeness of the calculated values of S, g_m and v_o/v_c obtained in this study to those published using fundamentally different methodology, lend support to photorespiration as the only other major sink for electron transport products in tobacco leaves.

We conclude that: (1) in transgenic tobacco with a decreased PRK activity and RuBP concentration the significant 'down-regulation' of PS II, measured at quantum flux above that encountered during growth, is the primary regulatory process enabling a balance between electron transport and carbon metabolism to be achieved, (2) the relationship between Φ_{CO_2} and $\Phi_{PS\,II}$ is similar in wild-type and transformant but is obtained at different poises in the component processes of photosynthesis and (3) photorespiration is the second major sink for electron transport products after RuBP carboxylation in tobacco leaves with no evidence for other alternative sinks.

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