Dependence of Photorespiration and Photosynthetic Unit Sizes on Two Interdependent Nuclear Gene Factors in Tobacco

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Dedicated to Prof. Dr. Josef Straub *at the Occasion of His 70th Birthday*

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A new set of tobacco mutants was obtained by selfing a single variegated plant which emerged in a seed lot of *Nicotiana tabacum var. Consolation.* The seeds obtained from this mutant give rise to four phenotypes: variegated, yellow, yellow-green, and green seedlings. The green, yellow-green and yellow characters are due to two *interdependent* nuclear gene factors. The yellow-green phenotype is the homozygous (*aabb*) true breeding condition, whereas the green and the yellow phenotype are heterozygous (*AaBb*) with respect to both nuclear factors, the difference in the yellow and green phenotype being the addition of a labile gene factor pair, *Cc*, in the yellow condition.

If photorespiration is measured as the *Warburg effect* or as $^{18}O_2$-consumption by mass spectrometry it appears that the heterozygous green phenotype is the defective condition with high photorespiration. The three phenotypes differ with respect to chlorophyll content and photosynthetic unit sizes, the photosynthetic unit size in the yellow phenotype being approximately 1/10 of that of the green type. The gene expression for photorespiration (measured as $^{18}O_2$-uptake for example) in the heterozygous green type is suppressed by the addition of the labile gene factor pair *Cc* in heterozygous condition which leads to the yellow phenotype. In the yellow and green phenotype the photosynthetic unit size is different but not the ratio of photosystem I/photosystem II activity.

Moreover, from the present studies it appears that the Warburg effect *i. e.* an increase of photosynthetic rate upon anoxia, can only partly be due to an inhibition of ribulose 1,5-biphosphate oxygenase or glycolate oxidase.

Modern agriculture and forestry are, to date, the main practices which exploit the conversion of solar energy into organic matter in an economic manner. However, these energy converting processes by no means function efficiently. Despite the fact that the photolysis of water and electron transport through photosystem II quickly reach an optimum, the assimilation of CO_2 is very wasteful in almost all plants. About 50% of the organic carbon compounds are lost in the process of respiration and photorespiration. In C_3-plants in which photorespiration plays an important role, distinct and considerable differences of various photorespiratory characteristics can be found for strains and mutants within a given plant species *e. g. N. tabacum* [1–3]. Depending somewhat on which reaction is taken as a measure of photorespiration (*e. g.* $^{18}O_2$-consumption) such differences can comprise up to one order of magnitude. Mutants exist in which the major proportion of the photoassimilate is wasted almost immediately after its formation. Thus among the C_3-plants, clearly, variants may have high or low photorespiration, in contrast to some reports and concepts in the literature [4], but fully in line with findings from other laboratories [2, 5, 3]. The possibility thus arises that in order to improve on crop yields it may be useful to characterize genetic factor combinations which affect photorespiration. Okabe *et al.* have recently shown that in a genetic situation where two independent nuclear gene factors were involved, both the size of the photosynthetic light antenna and photorespiration were affected [2]. It became evident that in the haploid condition the gene combination *Su/Aur* was characteristic of a high rate of photorespiration, whereas *su/Aur* was not [1]. In the normal tobacco plant condition the gene constitution *Su/su/Aur/aur* correlates with a high rate of photorespiration, whereas *Su/su/Aur/aur* or *su/su/Aur/aur*

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Abbreviations: C_2 3A: Cellules de Culture Automatique sous Atmosphères Artificielles.

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does not. In the present paper we have selected a mutant or plant set in which two interdependent nuclear gene factors are characterized with respect to their effect on photorespiration and also with respect to the size of the light antenna *i.e.* their photosynthetic unit size.

Materials and Methods

Plant material. The tobacco variety *Consolation* is a tobacco with a so-called yellow-green character due to a recessive gene pair *yg/yg*. This tobacco has been described in detail by Nolla [6]. The mutant set described in this paper is derived from a single variegated plant observed in a seedlot of Nolla's selfed *Consolation*. If not specified otherwise all plants were grown in the greenhouse.

Growth of the plants in an automatic growth chamber under an artificial atmosphere. The growth chamber, of the type C_2 3A (Cellules de Culture Automatique sous Atmosphères Artificielles) has been described in detail by André *et al.* [7]. The three tobacco phenotypes used were subjected to the same low (1–2%) – normal 21% oxygen pressure régime. After a period of normal oxygen partial pressure (21%), usually 2 days, the oxygen pressure was lowered to 1–2% by gassing the cabinet with oxygen-free nitrogen for 1½ h. The CO_2 partial pressure was then increased to 0.03% and the experiment started. After 1½ days the oxygen partial pressure was restored to normal in order to determine whether the photosynthesis rate observed before flushing with nitrogen remained changed or not. After usually a 3 day period under normal pressure, flushing with nitrogen was repeated in order to determine the reproducibility of the Warburg effect. The second period of anoxia generally lasted 3–5 days. Although technically possible, the low oxygen partial pressure of 1% was not purposely further lowered in order to avoid affecting stomatal control.

$^{18}O_2$-Uptake was measured in a MAT CH4 mass spectrometer with whole leaves or leaf sections in a closed gas circuit at the compensation point [8].

O_2-Uptake or -evolution was measured in the three-electrode system described by Schmid and Thibault [9].

Chloroplast preparations were prepared according to Homann and Schmid [10].

Activity of glycolate oxidase and ribulose biphosphate oxygenase was determined in crude extracts essentially as in the assay systems described by Schmid [11], Codd and Schmid [12], and Okabe *et al.* [2] using a Clark type electrode (Rank Brothers, Bottisham, England). Special attention was paid to the activity of polyphenol oxidase which interferes with O_2-uptake due to glycolate oxidase and ribulose biphosphate oxygenase on the electrode. As described by Koivuniemi and Tolbert, polyvinyl-pyrrolidone proved to be useful in eliminating polyphenol oxidase activity [13]. Also, as described by Tolbert [14] and for unknown reasons, the plants in the automatic growth chamber always appeared to have a higher polyphenol oxidase activity than greenhouse-grown plants.

Results

Characterization of the plant material

The mutants described in this paper were derived from a variegated plant which was observed in a seedlot of selfed *N. tabacum var. Consolation* (Fig. 1). Selfing of this variegated plant yielded 4 types of seedlings namely green, yellow-green, yellow and variegated ones (Fig. 2). From Tables I and II it is clearly seen that the property of being a variegated plant is controlled by the cytoplasm, whereas the properties green, yellow-green and yellow are controlled by nuclear gene factors. The usual crossings between green, yellow-green and yellow plants show that two interdependent nuclear gene factors (*A, B*) are involved. The seedling ratios in the crossings green × green, or green × yellow-green are very close to the ratio 7:9 which according to classical student text books [15] indicates interdependency of the gene factors involved (Table II). The yellow-green condition is homozygous with respect to at least one of the two factors (see also Fig. 4), whereas the green plants are heterozygous. The fact that yellow plants are observed in substantial numbers only when yellow plants are selfed, together with the result that some yellow plants are observed in the crossing yellow × green (Table II) shows that a labile nuclear gene factor pair must be added in a heterozygous condition to the green condition to give yellow plants. Thus, the three types of plants yellow, yellow-green and green have the gene constitution shown in Fig. 3. This gene constitution is due to the fact that the plants (green, yellow-green and yellow) which we used for all our physiological tests always

Fig. 1. Variegated tobacco plant isolated from a seed-lot of selfed *N. tabacum* var. Consolation with yellow-green character.

originated from the crossing yellow × yellow. It can be seen from the results in Table II that 5 types of yellow-greens and 4 types of greens exist, with the combinations shown in Fig. 4.

Photorespiration in the green, yellow-green and yellow phenotype

a) Warburg Effect: Whole plants of comparable sizes of the 3 phenotypes were grown in a growth cabinet in which gas pressures of CO_2 and O_2 were regulated and maintained (see Materials and Methods). Photosynthesis was measured in these plants as CO_2-fixation. At regular intervals the plants were subjected to a low oxygen partial pressure (1%) environment and photosynthesis measured. According to the literature a low oxygen partial pressure would be expected to reduce apparently wasteful oxidative enzymic processes including glycolate oxidation [16, 17] and the ribulose 1,5 biphosphate oxygenase reaction [18], thereby increasing net photosynthesis. This effect of anoxia on photosynthesis is called the Warburg effect [19]. The three phenotypes responded to low oxygen partial pressure in completely different ways. The quantitative enhancement effect on photosynthesis was the largest in the green phenotype (Fig. 5). Thus, apparent photosynthesis was stimulated from 2.4 liters of CO_2 to 4.5 liters taken up by the green plant (Fig. 5) representing an 87.5% increase in net photosynthesis. The return to normal partial pressure apparently restored

Fig. 2. Tobacco plants obtained from a seed-lot of the selfed variegated plant shown in Fig. 1. The seed-lot yields variegated, green, yellow-green and yellow cotyladones. The plants are 6 weeks old.

Table I. Seedling ratios in seeds from crossings between green, yellow-green, yellow and variegated tobacco plants from *N. tabacum* var. Consolation.

Crossing	Green	Yellow Green	Yellow	Variegated
Variegated × variegated	30	312	38	36
Variegated × yellow	0	144	132	4
Yellow × variegated	56	376	12	0
Variegated × green	136	232	40	84
Green × variegated	108	324	4	0
Variegated × yellow-green	44	232	36	108
Yellow-green × variegated	8	292	8	0

Table II. Seedling ratios in seed from crossings between green, yellow-green and yellow tobacco plants from *N. tabacum* var. Consolation.

Crossing	Green	Yellow Green	Yellow	Variegated
Green × green	234	144	0	0
Yellow-green × yellow-green	0	384	0	0
Yellow × yellow	28	330	14	0
Green × yellow-green	340	448	0	0
Green × yellow	376	460	0	0
Yellow × green	572	908	3	0
Yellow-green × green	84	124	0	0
Yellow-green × yellow	0	672	0	0
Yellow × yellow-green	0	1156	0	0

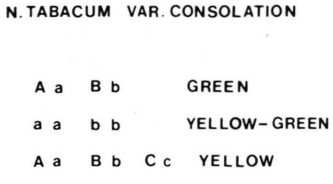

Fig. 3. Gene constitution of the green, yellow-green and yellow phenotype of *N. tabacum* var. Consolation used in this investigation.

Fig. 4. Possible gene combinations in the yellow-green and green phenotype of *N. tabacum* var. Consolation.

the original rate and the plant continued to grow (shown by a daily increase in the rate of photosynthesis). The second period of anoxia starting at the end of the 6th day increased the rate of apparent photosynthesis from 3.2 liters to 6.2 liters of CO_2, resulting as found in the first period in almost a 100% increase. In the second anoxia period which as described in Materials and Methods lasted longer than the first it can be seen that the high rate of apparent photosynthesis begins to decrease after the first day suggesting the participation of an adaptive process. This interpretation is substantiated by the observation that the apparent rate of photosynthesis after restoring the system to normal oxygen pressure on the 10th day is lower than expected and that the plants grew much slower than before that period, as evidenced by a reduced daily increase in the rate of photosynthesis (10th to 14th day).

When the same sequence of events was applied to the yellow phenotype, the responses were completely different (Fig. 6). First, the Warburg effect in the first period of anoxia was only 30–40% but the effect of the period of anoxia on plant metabolism was seen immediatily. After reestablishing normal

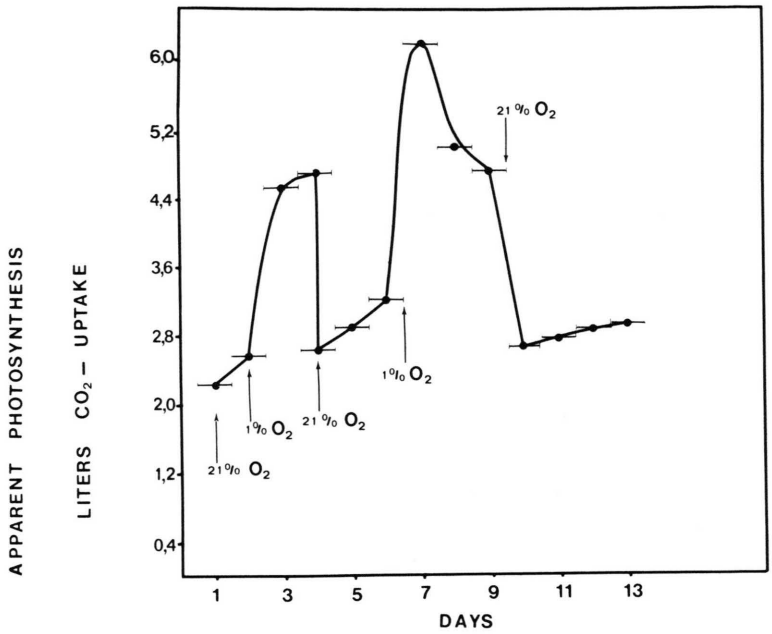

Fig. 5: Warburg effect on whole plants of the green phenotype *AaBb* in an automatic growth chamber. Changes in oxygen content in the growth chamber atmosphere are indicated by arrows. Temperature: 27 °C; Day length: 14 h. Plant size corresponding to approx. 500 cm² leaf surface. Bar length of points corresponds to 14 h. The arrow position indicates the time of oxygen partial pressure change.

oxygen partial pressure on the 4th day the rate of apparent photosynthesis had decreased by 30% and the plant growth had ceased. Apparently a process had been induced during the anoxic period which counteracts photosynthesis (measured as CO_2 fixation!) and which causes a daily decrease of the apparent rate of photosynthesis (4th to 7th day). The Warburg effect was quantitatively repeated during the second anoxic period but again a decrease in the rate of photosynthesis was found after a 1 day period. When the yellow-green phenotype was subjected to this high-low oxygen partial pressure treatment, a in general 30–40% Warburg-effect was observed and the anoxic period did not significantly influence the rate of apparent photosynthesis after restoring the system to normal partial pressures. Occasionally, however, even with this phenotype high Warburg effect were observed. The difference

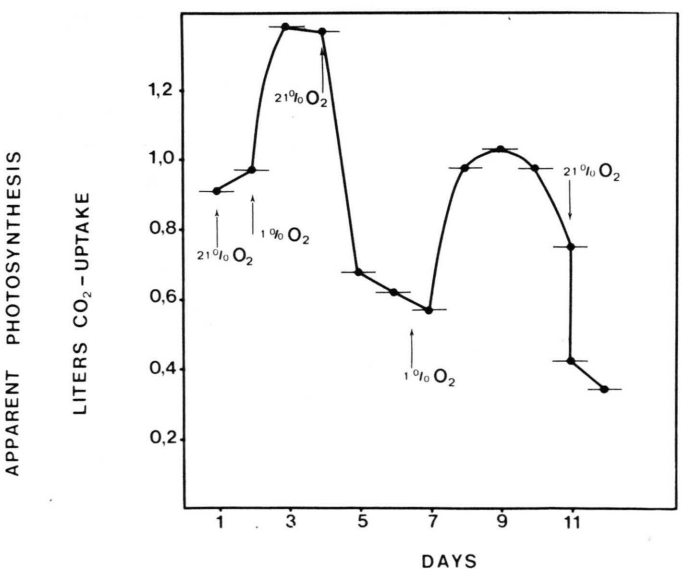

Fig. 6. Warburg effect on whole plants of the yellow phenotype *AaBbCc*. Conditions as in Fig. 5. Bar length of points corresponds to 14 h. The arrow position indicates the time of oxygen partial pressure change.

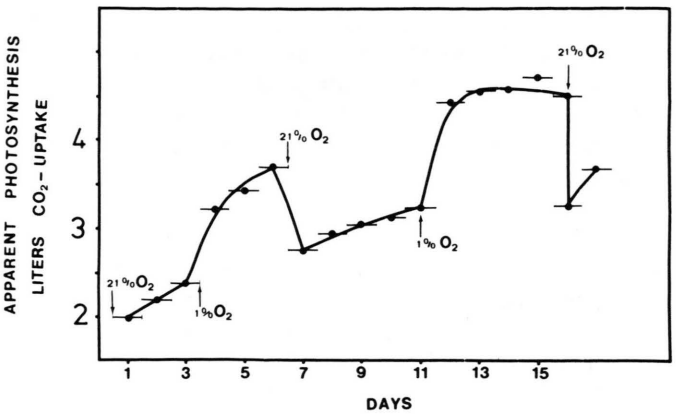

Fig. 7. Warburg effect on whole plants of the yellow-green phenotype *aabb*. Conditions as in Fig. 5 and 6. Bar length of points corresponds to 14 h. The arrow position indicates the time of oxygen partial pressure change.

in comparison to the green and yellow phenotypes was always (even under the condition of an exceptionally high Warburg effect) that the high rate of photosynthesis remained constant during the anoxic period and did *not* decrease as with the yellow or green phenotype (Fig. 7).

If the Warburg effect is taken as a measure of photorespiration then the green and yellow phenotypes exhibit on the average higher photorespiration than the yellow-green form. The largest effect *per se* under low oxygen partial pressure was seen with the green plants, but the results of the anoxic period upon plant growth were most pronounced with yellow plants. Hence, at least two effects of low oxygen partial pressure on the apparent rates of photosynthesis must be distinguished: A direct effect which leads to what is widely termed the Warburg effect. In addition, it appears as if enzyme affinities towards oxygen may be increased under low oxygen partial pressures which persist when the system is brought back to normal oxygen partial pressure and which are, under these conditions, disadvantageous to the plant.

b) $^{18}O_2$-Uptake: Probably the best way of estimating what is called photorespiration is by measuring $^{18}O_2$-uptake in the light by mass spectometry [8, 20]. As pointed out earlier a discrepancy does exist concerning the stoichiometry between the CO_2-burst and $^{18}O_2$-uptake in the light [9], the latter being larger by at least 1 order of magnitude than the rates of the so-called postillumination burst of CO_2 [17]. If this $^{18}O_2$-uptake is measured in the light with leaves of the three tobacco phenotypes, it appears that the green type has the highest $^{18}O_2$-uptake on a leaf area basis (Table III), supporting our previous findings that the green phenotype with the gene constitution

Table III. $^{18}O_2$-Uptake by leaves of the three phenotypes from *N. tabacum* var. Consolation.

Phenotype	$^{18}O_2$-Uptake [$\mu mol \cdot h^{-1}$]		CO_2 Compensation point	$^{16}O_2$-Evolution [$\mu mol \cdot h^{-1}$]		CO_2-Fixation [$\mu mol \cdot h^{-1}$]	
	per Chl	per cm^2	µl	per Chl	per cm^2	per Chl	per cm^2
Consolation green	228 ± 90	5.6 ± 2.4	66	127 ± 15	3.1 ± 0.4	154 ± 5	3.5 ± 0.5
Consolation yellow-green	357 ± 89	4.2 ± 1.1	50	353 ± 65	3.74 ± 0.6	400 ± 113	4.8 ± 1.2
Consolation yellow	1966 ± 320	4.2 ± 1.3	46	1720	3.5	1962	3.96

The values for the yellow and yellow-green phenotypes are averages of 6 determinations. The value of the green phenotype is the average of 4 determinations. The compensation point is the average of 5 determinations in all cases. Variations are above all introduced by leaf thickness and/or by the different chlorophyll contents due to leaf thickness. The variations given are absolute maximal variations. $^{16}O_2$-evolution and CO_2-fixation are the rates observed immediately after CO_2-injection into the system. $^{18}O_2$-uptake is the steady state rate at the respective CO_2-compensation point.

AaBb has the highest rate of photorespiration, whereas the yellow-green phenotype shows the lowest rate. It is of interest to compare also the rate of photosynthesis measured as $^{16}O_2$-evolution and CO_2 fixation with the $^{18}O_2$-uptake capacity (Table III). It should be noted again that for technical reasons $^{18}O_2$-uptake was measured in the presence of 40–70 ppm CO_2 which was the compensation point of the respective mutant. These results suggest that the green phenotype was the defective mutant exhibiting an especially high photorespiration. This is further substantiated by the fact that the CO_2 compensation point of the green phenotype was particularly high (Table III).

For comparison purpose the $^{18}O_2$-uptake values for the earlier described tobacco mutants *Su/su* [1, 3] and *Su/su var. Aurea* are given in Table IV. The data confirm earlier results by our laboratory and by Zelitch and Day [3] showing that *Su/su* exhibits an unusually high photorespiration when compared to the wild type John Williams Broadleaf (JWB).

Activity of glycolate oxidase and ribulose 1,5-biphosphate oxygenase in tobacco leaves

Glycolate oxidase and ribulose 1,5-biphosphate oxygenase activities were measured in extracts prepared from the plants used for studies on the Warburg effect (Fig. 5–7). When a tobacco plant was kept under anaerobic conditions it was found that glycolate oxidase activity quickly disappeared from its leaves and was barely detectable after a 2 day period of anoxia (Table V). The K_m of the enzyme remained unchanged throughout indicating that the functioning enzyme disappears and that the substrate adaptation of the enzyme observed earlier by Tolbert [21] is not only valid for glycolate but also for the other substrate oxygen. At first the activity of ribulose 1,5-biphosphate oxygenase, for ribulose 1,5-biphosphates as substrate, seems also to disappear under anaerobic conditions. A Michaelis-Menten plot for crude extracts of leaves, kept under anaerobic conditions yields sigmoidal curves which are difficult to interpret but which provide some indication of a lesser affinity of the enzyme towards oxygen. It should be noted that under conditions when these enzyme activities are barely detectable in extracts of the leaves, the Warburg effect, *i.e.* the enhancement of photosynthesis is nearly optimal. The authors feel, therefore, that the Warburg effect is only to a minor extend due to the inhibition of glycolate oxidase activity or ribulose 1,5-biphosphate oxygenase activity under anaerobic conditions. When the amount of glycolate was measured, for example, in leaves of the yellow-green phenotype it was found in the first short anoxia period (Fig. 7) that the amount of glycolate present was diminished (Table V), suggesting the inhibition of glycolate production by an inhibition of ribulose 1,5-biphosphate oxygenase activity in the absence of oxygen, as known from the literature [18]. However, in the second, longer, anoxia period, the amount of glycolate was appreciably increased (Table V) which could be interpreted as being due to an adaptative process in which the oxygenase under 1% oxygen has become adapted to the low oxygen partial pressure and functions again.

Photosynthetic unit sizes and electron transport properties in the three phenotypes

The three phenotypes differ with respect to their chlorophyll content per leaf area (Table VI). The yellow plants contain only 1/10 of the chlorophyll content of the green phenotype per unit leaf area at practically all growth stages. The chlorophyll a/b ratio seems to be the same in all three plants under

Table IV. $^{18}O_2$-Uptake, $^{16}O_2$-evolution and CO_2-fixation by leaves of the three phenotypes from *N. tabacum* var. John William's Broadleaf (JWB).

Phenotype	$^{18}O_2$-Uptake [μmol · h^{-1}]		$^{16}O_2$-Evolution [μmol · h^{-1}]		CO_2-Fixation [μmol · h^{-1}]	
	per Chl	per cm^2	per Chl	per cm^2	per Chl	per cm^2
JWB (Wild Type)	129	4.2	125	4.1	189	6.2
Su/su	619	5.6	1123	10.1	1865	16.7
Su/su var. Aurea	917	2.0	1189	2.8	1500	3.57

Conditions as in Table III.

Table V. Glycolate oxidase activity and glycolate content in leaves of *N. tabacum* var. Consolation yellow-green kept under normal and 2% oxygen partial pressure.

	Glycolate oxidase activity	K_m [M]	Glycolate content [nmol]	
	μmol O_2/min		per 52 cm² leaf	per mg Chl
Normal O_2-pressure (control)	0.45	1.17×10^{-4}	366 + 30[a]	457
2% O_2 for 1½ days	0.044	1.10×10^{-4}	441 ± 8[b]	323
Followed by 2 days normal O_2-pressure and by 5 days 2% O_2	0	1.10×10^{-4}	4062 ± 133[c]	6551

Glycolate content was determined in a Varian Mass Spectrometer, Modell MAT 112S. Glycolate oxidase activity was measured in the range of linear dependency of the enzyme on the amount of glycolate added. The activity was measured as O_2-uptake with a Clark type electrode upon the addition of 0.5 ml of crude leaf sap. The leaf samples[a, b, c] contained 0.741, 1.366 and 0.62 mg of chlorophyll.

Table VI. Pigment content in leaves of the tobacco mutants from *N. tabacum* var. Consolation.

Phenotype	Chlorophyll content per leaf area				
	Chla	Chlb	Total Chl	Chla / Chlb	Total carotenoid / Total chlorophyll
		[μg/cm²]		Ratio	
Young leaves					
Consolation green	11.18 ± 0.812	5.50 ± 0.161	16.68 ± 0.970	2.04 ± 0.146	0.2335 ± 0.0065
Consolation yellow-green	6.74 ± 0.994	2.29 ± 0.124	9.01 ± 1.118	2.90 ± 0.394	0.3010 ± 0.0137
Consolation yellow	1.69 ± 0.187	0.60 ± 0.029	2.29 ± 0.296	2.79 ± 0.246	0.5687 ± 0.0346
Fully expanded leaves					
Consolation green	17.90 ± 2.319	7.20 ± 0.882	25.10 ± 3.101	2.40 ± 0.187	0.2510 ± 0.0069
Consolation yellow-green	7.84 ± 0.906	2.58 ± 0.211	10.42 ± 1.107	2.89 ± 0.174	0.3369 ± 0.0129
Consolation yellow	1.70 ± 0.285	0.59 ± 0.061	2.29 ± 0.324	2.84 ± 0.476	0.6961 ± 0.0494

Table VII. Average size of the photosynthetic units and Hill reaction rates in isolated chloroplasts of the yellow, yellow-green and green phenotype of *N. tabacum* var. Consolation.

Phenotype	Green	Yellow-green	Yellow
Photosynthetic unit sizes [chlorophyll molecules/molecule O_2-evolved/flash]	1200 ± 60	610 ± 60	360 ± 60
Hill rate: $H_2O \rightarrow$ Ferricyanide [μmol ferricyanide reduced/mg chlorophyll/h]	200 ± 13	238 ± 18	2280 ± 198
Dark time (msec) between flashes necessary to obtain half optimal photosynthetic units at 22 °C	28	15	11

our growth conditions. Upon increasing the greenhouse temperature the yellow-green and especially the yellow phenotype increased their chlorophyll a/b ratio considerably. But as already described for the *Su/su* mutant [2, 10] the carotenoid/chlorophyll ratio was increased considerably in the chlorophyll deficient phenotypes. Despite their low chlorophyll content the yellow plants have a growth rate comparable to that of the green and yellow-green phenotypes, provided that light intensities and temperatures are high enough. The physiological properties are very similar to those described earlier for the *Su/su* and *Su/su var. Aurea* mutant [2, 10]. As detailed previously [2, 10], the yellow phenotype has a reduced photosynthetic unit size (Table VII). As a consequence of the reduced size of the light antenna, all electron transport reactions in the chlorophyll-deficient yellow phenotype are several-fold higher on

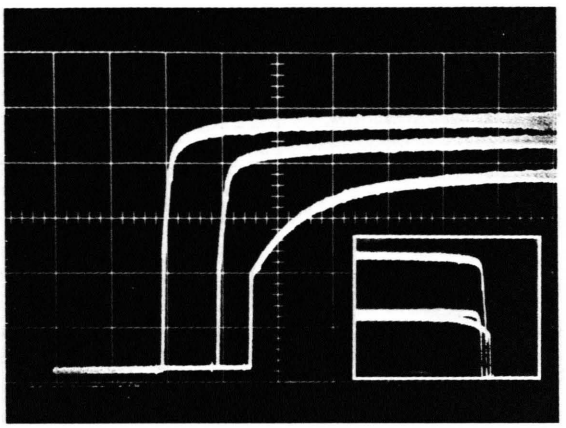

Fig. 8. Fluorescence induction at room temperature in tobacco chloroplasts. Assay: 3 ml; Tricine 0.03 M/KCl 0.06 M buffer. Chloroplasts corresponding to 6–7 μg chlorophyll/ml. Assay in the presence of 2×10^{-6} M DCMU and 4×10^{-3} M $MgSO_4$. Sweep speed: 0.1 sec/square. Sensitivity: 50 mV. 10 min dark adaptation before fluorescence excitation. Upper curve: green phenotype. Middle curve: yellow-green phenotype. Lower curve: yellow phenotype. Inset, early seedling stage: Upper curve: yellow-green phenotype. Middle curve: green phenotype. Lower curve: yellow phenotype.

a chlorophyll basis than in the green or yellow-green phenotypes (Table VII).

Fluorescence at room temperature is supposed to come exclusively from photosystem II [22]. We therefore compared the fluorescence induction behavior in the three phenotypes. Fig. 8 shows the induction curves for chloroplasts correspondings to 10 μg of chlorophyll for each of the three phenotypes. It is seen that the steady state fluorescence in the presence of DCMU is essentially the same on a equal chlorophyll basis in all three phenotypes, despite the fact that the unit size is distinctly different. The data indicate that the ratio of photosystem II/photosystem I is the same in all three tobaccos. In the early seedling stage the yellow-green phenotype, however, seems to have a higher photosystem II content per unit chlorophyll.

Discussion

The present study is a continuation of earlier work [1, 2, 23] in which we searched for genetic conditions or genetic factor combinations which correlate with an especially low, or especially high level of photorespiration. The plant we chose was tobacco because the selection of useful mutants from tobacco is particularly straight forward. A single plant produces approximately 100 000 seeds which can all be brought out in a single experimental sowing. In such a dense seedling population chlorophyll or variegated mutants are easily descernible by their color difference. Treatment with chemicals or simple aging of the seed lot increases the probability of finding mutants.

In the techniques of conventional and modern plant breeding it is by no means selfevident that the high yield selections will necessarily also show a minimal photorespiration. As evident from our earlier work [1, 2] and the present paper, the observation is made that two or more gene factors in the proper condition may, *among other* things affect, chlorophyll content *and* photorespiratory activity. Concerning the tobacco mutant *Su/su var. Aurea* [2], or the yellow phenotype of *N. tabacum var. Consolation* in this paper, it is likely that any conventional plant breeding would have eliminated these plants as being undesirable, since under normal conditions they would grow too slowly in comparison to the green control. On the other hand, the haploid plants with *Su/Aur* or *su/Aur* [1] or in the present paper *AB* or *aB*, may well provide material for modern methods of plant breeding [24]. The conclusion drawn from the present work is firstly that there is no doubt, that within one plant species *e.g. N. tabacum*, strains and mutants exist which differ with respect to their photorespiratory activity. Secondly, these differences in photorespiration can be genetically manipulated. These conclusions support data and interpretations of Zelitch's laboratory [3, 5, 17] and do not speak in favor of a pleiotropic effect. However, the importance of such a genetic manipulation in the light of the present paper is certainly already restricted by the simple fact that our observations refer to mutants and in particular to nuclear mutants. In this context a very recent paper by Yeoh *et al.* [25] on variations in K_m CO_2 of ribulose 1,5-biphosphate carboxylase among C_3 grasses is of special interest.

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