The Kok Effect and Its Relationship to Photorespiration in Tobacco

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The Kok effect of photosynthesis was investigated in different tobacco mutants. It was found that the breaks in the light intensity curve were always at or around 1000 lux in all plants tested regardless of the unit sizes which differed by a factor of 10. It was concluded that the photoreceptor responsible for the effect must be present in the wild type and the chlorophyll deficient mutants in the same amount and is probably not chlorophyll. Due to the fact that the light dependency of the Hill reaction in isolated tobacco chloroplasts also shows a break at or around the "Kok intensity" it was concluded that probably a structural change of the photochemical apparatus around 1000 lux contributes to the effect. Measurement of $^{18}O_2$-uptake by mass spectrometry at low light intensity shows at low CO_2-concentration an enhancement of $^{18}O_2$-uptake again at/around 1000 lux indicating that photorespiration starts to function at the "Kok intensity". Due to the fact that $^{18}O_2$-uptake remains constant at high CO_2-concentrations the break in the photosynthetic light intensity curve cannot be due to an inhibition of "dark respiration" at low light intensities.

In recent years we have tried to elucidate the phenomena of photorespiration by studying mutants with different photorespiratory activity [1, 2]. In this context low light intensity phenomena such as the Kok effect became interesting. In 1949 Kok [3] observed a break in the photosynthetic light intensity curve of *Chlorella* which was confirmed by Van der Veen for tobacco [4]. According to the studies with some species of higher plants by Ishii *et al.* [5–7], the Kok effect is one of the photosynthetic characteristics of C_3 plants having a breaking point at around 1000 lux. The question still remaining until this day is whether respiration is inhibited at low light intensities, whether photosynthesis is stimulated at low light intensities, or whether at the breaking point another CO_2-evolving process such as photorespiration starts to function.

In order to solve the question whether the Kok effect has anything to do with photorespiration we attempted to investigate the capacity of tobacco plants at and around the "Kok light intensity" for $^{18}O_2$-uptake by mass spectrometry and compare these results with the capacity of Hill activity of isolated chloroplasts of these plants at or around the breaking point.

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Materials and Methods

Plant material: The tobacco mutant *Su/su* was discovered by Burk and Menser [8]. In a seed lot of a selfed *Su/su* plant a variegated plant was observed which yielded *Su/su* var. *Aurea* [1].

The mutants *Su/su* and *Su/su* var. *Aurea* originated from an aged seed lot of the Connecticut cigar variety John William's Broadleaf (JWB). The tobacco mutant Consolation is due to a recessive gene pair (yg/yg) which was described by Nolla [9]. In a selfed seed-lot of this mutant type, again a variegated plant was isolated which upon selfing yielded "Consolation green", "Consolation yellow-green", "Consolation yellow" and variegated plants [10]. All these plants were green-house grown with the growth conditions described in a previous paper [1].

Measurements of photosynthesis as CO_2-fixation were carried out in a flat plexiglass chamber ($25 \times 20 \times 0.7$ cm³) in which a cut out leaf, standing in water was flushed with normal air at the rate of 1 litre per minute, with the CO_2 measurement done with a URAS (Hartmann and Braun). The desired temperature was obtained by regulating the temperature of water in which the leaf chamber was immerged. Humidity was kept constant at around 80% by passing the air flow through thermostated water.

The leaf was illuminated by means of a photoprojector (Prado Universal, Ernst Leitz GmbH, Germany).

Hill reaction rates in chloroplasts were measured with a Clark type oxygen electrode (Rank Brothers, England) with the chloroplasts (25–50 µg chlorophyll) prepared according to Homann and Schmid [11] in 5 ml assay medium (pH 7.8) involving 1 mM K_3 [Fe(CN)$_6$] 50 mM Tricine, 10 mM NaCl, 3 mM $MgCl_2$, 1.5 mM K_2HPO_4, 1.5 mM ADP for the coupled condition, and 1 mM K_3 [Fe(CN)$_6$] 50 mM Tricine, 10 mM NaCl, 1 mM NH_4Cl for the uncoupled condition. The reaction was started by illumination.

$^{18}O_2$-Uptake was measured in a MAT C-1144 with leaf sections of around 100 cm² in a closed gas circuit according to Dimon *et al.* [12].

Results

In the following studies we used the tobacco mutant set which originated from the Connecticut cigar variety John William's Broadleaf (JWB) and which is one of the best investigated higher plants with respect to photorespiration [1, 2, 13]. In addition we have used the tobacco mutant set which originated from Nolla's Consolation and which has been characterized very recently [10].

CO_2 exchange: If the photosynthetic light intensity curves in the low light intensity region are plotted, a Kok effect is easily detected with any of these plants. Regardless of what unit size the mutants have, the break of the light intensity curve is at or around 1 000 lux. Fig. 1 shows the Kok effect for leaves of JWB and *Su/su* under comparable conditions. Clearly, *Su/su* shows the break at the same light intensity as JWB despite the fact that its photosynthetic unit is roughly 5 times smaller than that of JWB [14]. Thus, the photoreceptor for the observed phenomena must be present in *Su/su* and JWB in the same absorbing or effective amount per surface leaf area. Essentially the same situation is observed if *Consolation green* or *Consolation yellow-green* are compared (Fig. 2), which are plants that differ with respect to their photosynthetic unit sizes [14, 15]. Also, the size of the Kok effect itself does not seem to be considerably different between the tobacco variants, which confirms what has been said above and shows moreover that chlorophyll cannot be the primary photoreceptor. Since the effect is seen in the light intensity curve of photosynthesis an auxiliary pigment might be a candidate.

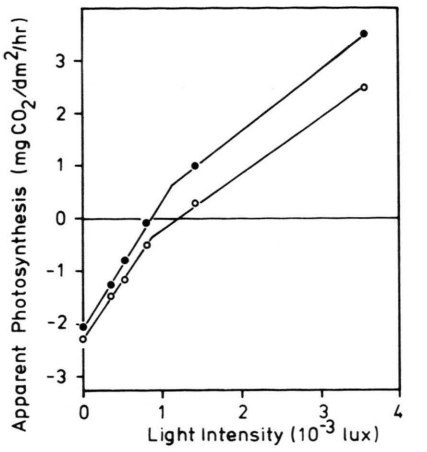

Fig. 1. Photosynthetic light intensity curve of JWB (●) and *Su/su* (○).

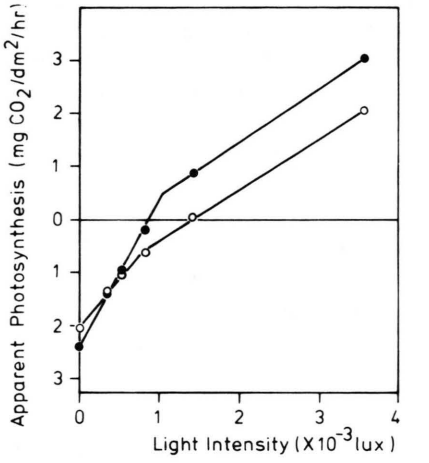

Fig. 2. Light intensity curves of the tobacco mutant Consolation green (●) and Consolation yellow-green (○).

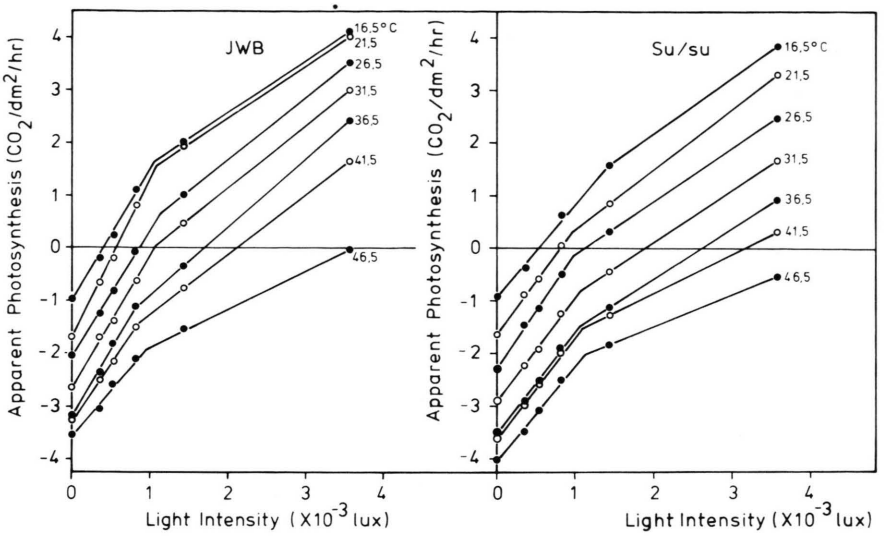

Fig. 3. Temperature dependence of the Kok effect in the tobacco variety JWB and the mutant *Su/su*.

The Kok effect has a slight temperature dependence (Fig. 3) which is also comparable in the different tobacco mutants. At higher temperatures the breaking appears to be shifted to lower light intensities. As photosynthesis in this light intensity region is insensitive to temperature, the Kok effect is apparently due to an enzyme limited reaction sequence which either starts its action at the Kok intensity or which changes its activity at the discussed light intensity around 1000 lux.

Hill reaction: Photosynthesis in low light intensity is regulated by the activity of purely photochemical reactions which are insensitive to temperature. The temperature dependency of the Kok effect in tobacco was very small in contrast to that in rice plants (Fig. 3). This result led us to the idea that there might be a sort of Kok effect in the photochemical reaction itself. Fig. 4 shows the Hill activity of islolated tobacco chloroplasts, in which a clear break of the light intensity is observed in the Kok intensity region. The higher rate of photosynthetic electron transport at the light intensity below the break is not due to better coupling of electron transport to photophosphorylation since phosphorylating or uncoupling conditions did not influence appreciably the break in the light intensity curve. If the phenomena shown in Fig. 4 play a role in the intact leaf, only a structural change of the photosynthetic apparatus induced by light can be responsible.

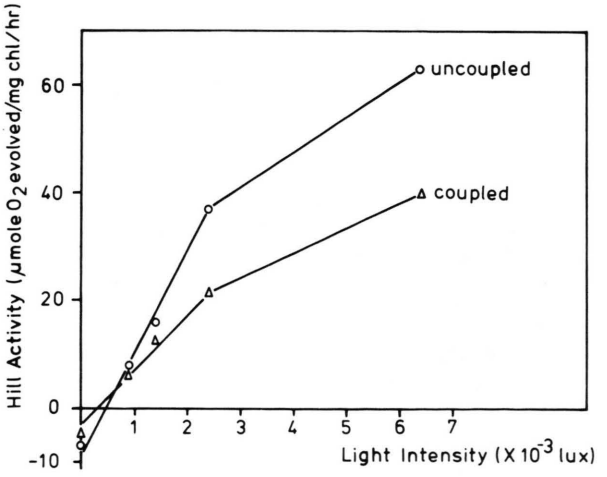

Fig. 4. Hill activity of JWB chloroplasts at low light intensities under coupled and uncoupled conditions at 25 °C. Each value is the average of three measurements.

Mass spectrometry: $^{18}O_2$-uptake: Probably the best way of estimating what is called photorespiration is by measuring $^{18}O_2$-uptake in the light by mass spectrometry [12]. The $^{18}O_2$-uptake measurements were carried out for technical reasons in a closed circuit. In high CO_2 concentration (0.2%) where according to the literature [16] photorespiration is inhibited, the $^{18}O_2$-uptake was constant in the region of the Kok intensity (Fig. 5). From this it can be concluded that dark respiration under these conditions is not influenced by light. In low CO_2 (at the CO_2 compensation concentration) where photorespiration is active according to the literature [16, 17], the $^{18}O_2$-uptake was strongly accelerated at around 1 000 lux (Fig. 5) which could mean that the Kok

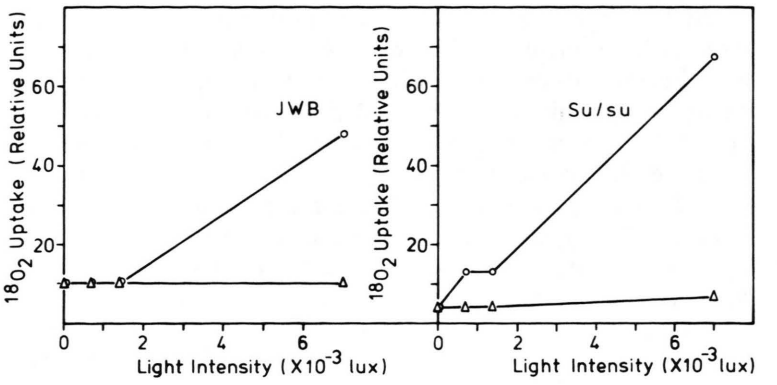

Fig. 5. $^{18}O_2$-uptake by a leaf section of the tobacco variety JWB and the *Su/su* mutant in dependence on light intensity. (○) low CO_2-concentration (0.005%) (△) high CO_2-concentration (0.2%).

effect is part of a photorespiratory phenomenon and that photorespiration (when measured as $^{18}O_2$-uptake) starts to function at the Kok intensity.

Discussion

In the present paper we demonstrate that the low light intensity induction phenomenon of photosynthesis known as "Kok effect" is somehow involved in photorespiration. This statement is based on measurements of $^{18}O_2$-uptake and CO_2-fixation. In this context we would like to draw the readers attention to the actual controversial stand points as to what significance photorespiration finally has. Andrews and Lorimer [18] and the Tolbert school think that photorespiration (measurable as gas exchange, opposite to the photosynthetic one in the light) is more or less constant in all C_3 plants and therefore unavoidable and not likely to be changed by genetic manipulation since it is an inherent property of the properly functioning CO_2-fixing enzyme system. On the other side Zelitch and co-workers [13] or Schmid and co-workers [1, 2] and others believe that photorespiration is a wasteful process which should better be genetically eliminated. These laboratories have repeatedly shown that photorespiration is dependent on certain genetic factor combinations. Okabe [19] has furthermore shown that in tobacco mutants which differ with respect to their photorespiratory activity the affinity of the enzyme ribulosebiphosphate carboxylase/oxygenase is changed towards its substrates. These observations gain new interest in the light of very recent observations by Yeoh *et al.* [20] in which the authors show that the Km of CO_2 of ribulosebiphosphate carboxylase *i. e.* the photosynthetic efficiency at low CO_2-concentrations in C_3-grasses varies widely.

All these seemingly incoherent standpoints and observations show that what is called photorespiration is a composite phenomenon, with several types of reactions involved. Thus, by comparison of O_2-uptake measurements ($^{18}O_2$-uptake by mass spectrometry) with those of the CO_2-postillumination burst one can easily show that O_2-uptake in photorespiring species exceeds by far the CO_2-evolution phenomenon [21]. The ribulose biphosphate carboxylase/oxygenase function or glycolate oxidase activity contributes under certain conditions only little to the overall oxygen-uptake phenomenon in the light. In this context it was shown that some of the strongly photorespiring species such as the *Su/su* tobacco mutant dispose of excess reducing power which is apparently generated by a very active photosystem II, by exhibiting a very rapid O_2-uptake which is probably due to a Mehler type reaction [22]. In this case the action spectrum is obviously the spectrum of chlorophyll [21].

In the present paper, however, one can show that the photosynthetic light intensity curve measured as CO_2-fixation (Figs. 1–3) has the Kok break at the same intensity where $^{18}O_2$-uptake, hence photorespiration, starts to intervene with photosynthetic O_2-evolution (Fig. 5). Due to the fact that the Kok effect has in the usual temperature region of 20–35 °C practically no temperature dependence (Fig. 3) and due to the observation that the light intensity curve of the ferricyanide Hill reaction of isolated chloroplasts also has a break at roughly the same light intensity we conclude that only a structural change in the photosynthetic apparatus in-

duced by white light at around 1 000 lux can be responsible for the effect. Moreover, due to the fact that mutants which differ with respect to their photosynthetic unit size *i. e.* their chlorophyll content by one order of magnitude, exhibit the Kok effect at the same light intensity, we conclude that chlorophyll is probably not a candidate for the photoreceptor. This refers to the reaction which causes the onset of photorespiration which is a low light intensity phenomenon and which must be dinstinguished from the high light intensity phenomenon photorespiration itself. It is evident that we will check whether blue light will be more effective in producing Kok effects than red light.

Our observation with the Kok effect further contributes to the notion that photorespiration is a complex phenomenon. An inhibition of "dark respiration" at low light intensities is excluded by our experiments.

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