CHLOROPHYLL FLUORESCENCE: a non-destructive method for detecting damage in the photosynthetic apparatus in plants

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Abstract

Various environmental factors inflict damage on the photosynthetic apparatus of plants, resulting in a decrease in productivity. Often this damage can not be detected by visual observations whereas many instrumental techniques are either destructive or less suitable for practical reasons.

Measurement of chlorophyll fluorescence on intact leaves allows for a rapid assessment of the "photosynthetic condition" of plants. Recently a new apparatus has become commercially available. It offers the opportunity to measure the ratio of minimal fluorescence in the dark and maximal fluorescence induced by high-intensity light flashes. A derivative of this ratio is a measure for the functioning of energy-transfer in the chloroplasts of intact leaves. The measurements take only a few seconds and it is possible to test a large number of plants, for instance in screening genotypical variation for stress tolerance or in measuring product quality.

On a time scale of minutes and in combination with gas-exchange measurements, fluorescence kinetics provide valuable information on the interaction between stress factors and fundamental photosynthesis processes.

The application of chlorophyll fluorescence measurements is illustrated by two case studies:

1 A fast detection method of cold damage in maize, applicable for breeding cold tolerant genotypes. A single measurement takes only a few seconds.

2 An application to detect the effects of water stress on primary photosynthetic reactions. A single measurement takes about 3 minutes.

1. Introduction

Abiotic stress factors lead to substantial losses of crop productivity and crop quality. Breeding of stress-tolerant plants as well as an early detection of the stress impact, are of great economical importance. However, from a physiological point of view, stress is an undefined condition due to the interference of many different processes in reaction to stress. For example, photosynthesis may be inhibited under a certain stress condition, causing a lower growth rate. But the other way around, a low photosynthetic rate may be due to a feedback mechanism, evoked by a low cell extension rate. In the latter case, attention should be focused primarily on the process

of cell extension and not on photosynthesis. This example illustrates that it is essential to define the stress induced rate-limiting processes.

1.1 Water stress

There are two conflicting views on the inhibition of photosynthesis due to water stress, i.e. stomatal and mesophyll limitation.

1.1.1 Stomatal limitation

According to the classical view, water stress induces closure of the stomata, either due to a low leaf water content or due to some other signal transduced to the leaves. The resulting increased resistance of the stomata for CO_2 diffusion causes a reduction of the CO_2 concentration inside the leaf, lowering the rate of photosynthesis (reviews by Bradford & Hsiao, 1982; Ceccarelli,1984). Thus stomatal limitation is reflected in a reduced internal CO_2 concentration and an increased share of the gas phase resistance to the total resistance for CO_2. Photosynthesis is less inhibited than transpiration because of an increased CO_2 gradient between interstomatal spaces and the ambient air, while that for water vapour remains the same. Water use efficiency, at given vapour pressure deficit, is therefore enhanced by water stress.

1.1.2 Mesophyll limitation

In addition to stomatal closure, water stress may reduce the photosynthetic capacity directly, either by inhibiting the Calvin cycle or the rate of electron transport over the chloroplast membranes (review by Kaiser, 1987). Stomatal aperture is also reduced, but only sofar that the internal CO_2 concentration remains unaffected (e.g. Wong et al.,1979, 1985). Thus stomatal aperture is tuned to the mesophyll limitation and both show a correlated response to water stress, probably mediated by abscissic acid (Schulze, 1986). The mechanism of mesophyll limitation is expressed by a constant ratio of CO_2(internal)/CO_2(external) and the share of gas phase resistance to total resistance remains unchanged when water stress occurs. Photosynthesis and transpiration are equally reduced and thus water use efficiency remains the same.

In vivo chlorophyll fluorescence distinguishes between stomatal and mesophyll limitation and can be applied to study changes in photosynthetic reactions provoked by water stress (Havaux and Lannoye, 1985; Schreiber and Bilger, 1985).

1.2 Cold stress

A similar approach may be applied in investigating aspects of cold tolerance in plants adapted to warm climatic conditions. Maize for instance, is very sensitive to low temperature damage, especially at high light intensity. The functioning of Photosystem II is blocked, which causes a decrease in the photosynthetic efficiency. The repair mechanism is relatively slow and therefore growth may be inhibited after alleviation of the low temperature stress. Photo-inhibition may thus limit growth, but it might as well be a protective mechanism against the excessive formation of free radicals that destruct chlorophyll by photo-oxidation. Maize seedlings, grown at low temperatures, have a low chlorophyll content (Alberda, 1969). Unfortunately there is no consensus about the causes (Wang, 1982) nor to what extent this suppresses growth (Miedema et al., 1987). In the

temperature range of 10 °C to 15 °C newly formed chlorophyll is presumably destroyed by rapid photo-oxidation before it is complexed in the chloroplast lamellae (Mc William and Naylor, 1967). At temperatures below 10 °C the effects are more pronounced because free radicals of oxygen destroy membrane-bound chlorophyll despite the protective action of carotenoids (van Hasselt and van Berlo 1980, Baker et al. 1983, Wise and Naylor 1987, Smillie et al. 1987). Alternatively, Hodgins and van Huystee (1986) showed that chill-induced chlorosis in maize seedlings was partly the result of two metabolic blocks in the porphyrin path leading to chlorophyll synthesis. The temperatures with impaired chlorophyll synthesis coincided with the temperature range for chlorosis (i.e. 17-10 °C).

In addition to the low temperature effect on the chlorophyll properties, low temperature influences metabolic and respiratory processes (Miedema et al., 1987). Thus low-temperature effects on growth are not necessarily due to photo-inhibition or photo-oxidation. The search for genetic differences for cold resistance should therefore take into account the different underlying processes. However, the procedures to identify genotypical differences at such level are complicated and time consuming. Analyses of chlorophyll fluorescence kinetics may elucidate the effects of low temperatures on fundamental photosynthesis processes and the extent of genetic variation. Absorbed light energy is used as driving force for the generation of chemical energy, it is dissipated as heat or it is emitted as fluorescence. Thus changes in one of these three processes will affect the other. In this study photo-inhibition, causing a deficient energy transfer from antennae pigments to photosystem II, is taken as a case study.

2. Material and methods

Intact leaves or leaf disks of both stressed and control plants were dark adapted for 20 minutes before the measurement. The leaves were clamped in a small cuvette, flushed with humidified air. Optical signals from and towards the leaves were transmitted by fibre optics. Fluorescence induction curves were recorded using a modulation fluorometer (PAM 101 Chlorophyll Fluorometer, H. Walz, Effeltrich, FRG).

2.1 Photoinhibition

Chlorophyll fluorescence was measured using leaf disks of maize. The disks were treated at low temperature and different light intensities in a screening apparatus depicted in Fig.1. After dark adaptation, the leaf disks were placed in the cuvette and fluorescence was excited by a measuring light beam (<680 nm). The intensity of the measuring beam was too low to excite photosynthesis. The outcoming fluorescence (>720 nm) is therefore called the "basic"-fluorescence (F_o). The measuring light (ML, fig. 2) was pulsed with a high frequency (100 KHz) and the induced fluorescence was detected. The fluorescence photodetection system detected only light of that frequency, thus preventing interference with background light-sources (Schreiber et al., 1985). A pulse of saturating light (SP) was given to close all reaction centres of photosystem II, which caused the fluorescence to rise to its maximum level (F_M). The ratio $(\Phi_{PC})=(F_M-F_o)/F_M$ gives the efficiency of energy transfer from antenna pigments to open reaction centres of photosystem II, which is directly related to the quantum

efficiency of photosynthesis. Damage due to photoinhibition is reflected quantitatively by a decrease of Φ_{PC}.

2.2 Fluorescence induction curves related to water stress

Five potato cultivars were grown on a nutrient solution in a conditioned glasshouse. Water stress was imposed by adding 20% polyethylene glycol to the nutrient solution. Photosynthesis, transpiration and chlorophyll fluorescence were measured using intact leaves during the stress period and after a recovery period. Changes in stomatal conductance can be derived from the measured transpiration rate and the water vapour pressure deficit but the mesophyll conductance is a complex parameter that is not easily accessible. It is mainly determined by the rate of electron flow from H_2O to NADP and by the Calvin cycle activity. Fluorescence induction kinetics may elucidate the mode of action of water stress on these processes.

Fluorescence induction kinetics were measured after activation of photosynthesis by an actinic light source (Fig.2, AL). The fluorescence increases rapidly because photosystem II is being reduced (closed traps). Subsequently photosystem I oxidizes photosystem II and the fluorescence is quenched. This quenching is called photochemical quenching (q_P). In addition there are quenching mechanisms not directly related to the electron transport: non-photochemical quenching (q_N), related mainly to a proton gradient across the thylakoid membrane (energy for ATP-formation) and the Calvin cycle activity (energy consumption).

A detailed discussion of the fluorescence quenching analysis is given by Schreiber & Bilger (1985). Both q_P and q_N alter continuously in the light. Saturating light pulses, superimposed on the actinic light, results in closing of all traps of photosystem II, giving the maximum fluorescence in the light; F_M'. The photochemical quenching changes during actinic illumination according to the ratio of open and closed photosystem II traps are mathematically expressed as:

$$q_P = (F_M' - F)/(F_M' - F_o').$$

The non-photochemical quenching q_N can be derived from the changes in maximum fluorescence and the basic fluorescence in the dark and the light respectively: $q_N = 1 - (F_M' - F_o')/(F_M - F_o)$.

3. Results and discussion

3.1 Fast screening for cold tolerance in maize

Maize is very sensitive to photoinhibition. A 4-hour treatment of maize leaf disks at 5 °C and 200 Watt/m^2 PAR caused a 50% decrease of the photosynthetic efficiency, Φ_{PC} (Fig.3). This damage, caused by a malfunctioning of photosystem II is proportional with light intensity and inversely correlated with temperature. For Dutch conditions this means that it is to be expected that the dry matter production in spring is considerably reduced by this phenomenon. Prolonged exposure to high light intensities, even at a relatively high temperature (Fig. 3b), reduces the photosynthetic efficiency considerable. Field experiments however showed that the effects were less severe. A monthly sampling of leaves of 75 maize accessions during the 1990 spring showed a reduction of Φ_{PC} by on average only 15-20%, though with considerable genetic variation. A possible explanation may be found in adaptation to low temperature under field conditions. Simulation of growth under

field conditions, using the photosynthetic efficiency as derived from the measured Φ_{PC}, showed that the overall effect of photoinhibition gave a 10-15% reduction in yield.

3.2 Effects of water stress on photosynthetic processes

Reduction of leaf photosynthesis due to water stress has been analyzed into various components for five potato cultivars. Water stress reduced photosynthesis, initially as a consequence of stomatal closure, but after 3 days increasingly by inhibiting directly the photosynthetic processes (mesophyll limitation). Stomatal closure correlated with the reduction in photosynthesis, but it was not the sole cause of this reduction because the internal CO_2 concentration in the leaves was not affected by water stress, indicative for other inhibitory factors. The time course of the q_P (Fig.4a), shows that the redox-state of photosystem II was not affected by water stress, except for day 9, when the temperature was increased from 17 to 27 °C. This suggests that the electron transport rate and the quantum efficiency were not affected by water stress solely but by a combination of water stress and high temperature.

The fluorescence signals related to the energy state of the chloroplasts ,i.e. the calculated q_N (Fig. 4b), show that the energy state of the chloroplasts increased as a consequence of water stress. The increased q_N is caused by an increase of the proton gradient due to a decrease of the ATP consumption in the Calvin cycle (Bradbury & Baker, 1983). Young leaves apparently suffered more than old leaves. The recovery after alleviation of stress, however, was also faster in young leaves than in old ones. The q_N on day 9 was not analyzed because the electron transport rate was severely impaired due to the change in temperature. This would bias the results because the inhibited electron flow itself will lead to a low proton-gradient. Fig. 5 shows the exponentially fitted relation between the calculated mesophyll conductances and q_N. The inhibited Calvin cycle apparently relates to an increase of the mesophyll conductance.

In young leaves recovered from stress, mesophyll conductance was greater than in leaves from the control treatment (0.33 versus 0.26 cm s^{-1}). Conductance of the gas phase however, was smaller (0.33 versus 0.53 cm s^{-1}) causing stomatal limitation, and so a reduced internal CO_2 concentration and increased water use efficiency. Apparently, photosynthetic capacity recovered faster than the regulation mechanism of the stomata. The resulting shortage of CO_2 thus leads to a relative surplus of reducing power and this might lead to the formation of free radicals of oxygen and the subsequent damage of photosystem II. Damage upon watering young potato plants after a drought period are known in practice and might be explained by this mechanism. Kaiser (1987) concluded from his results that rehydration of membranes caused a greater damage than partial dehydration.

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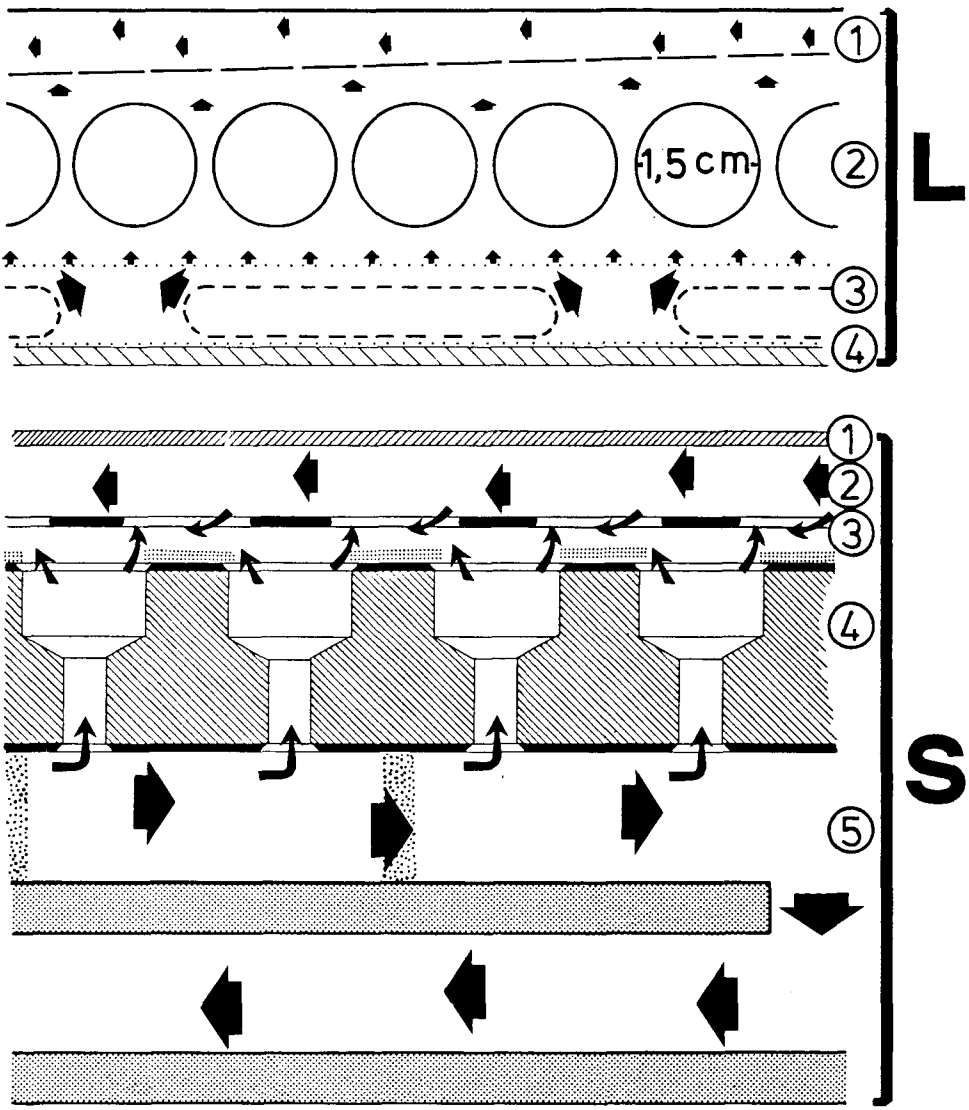

Figure 1 - Schematic cross section of experimental setup for photo-inhibitory treatment of leaf disks. L 1-4: Illumination setup with Fluorescent tubes (Phillips TL-33). Arrows indicate air movement for lamp cooling.

S1: Heat reflecting filter (Schott tempax 115).
S2: Temperature, humidity and speed controlled air movement.
S3: Layer covered by a perforated aluminium shield containing leaf discs (dotted bars).
S4: Temperature controlled roughly anodized aluminium block with air inlets (airflow 1-10 cm/s).
S5: Air pressure distribution plenum with moisture control.

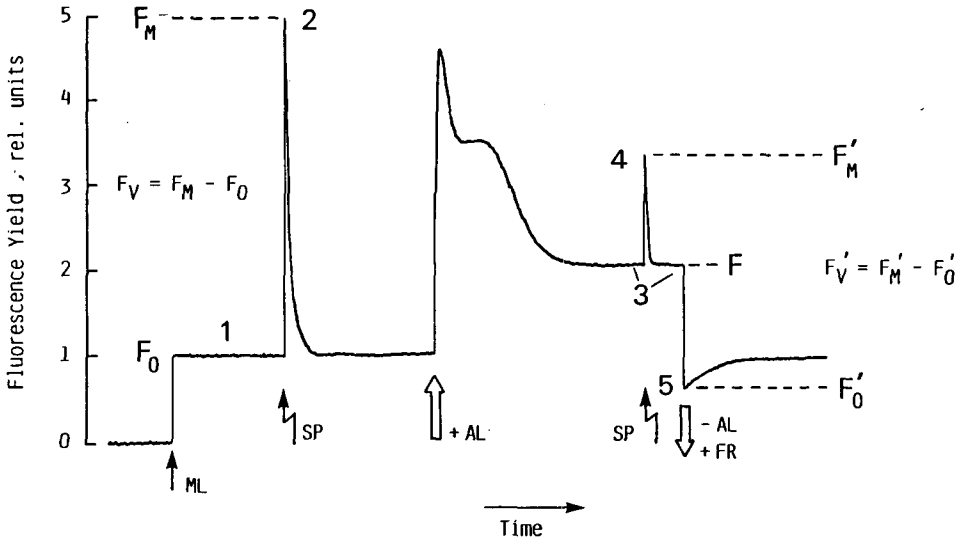

Figure 2 - Principles of fluorescence quenching analyses. The arrows indicate the switching of different light sources: ML=measuring light, SP= saturating light pulse, AL = photosynthetic active light, FR= far red illumination to oxidize photosystem II. Fluorescence parameters are indicated as: F_M, F_M' (maximum fluorescence in the dark and the light); F_o, F_o' (minimum fluorescence in the dark and the light); F_V, F_V' (variable fluorescence in the dark and the light).
q_P = photochemical quenching; q_N = non-photochemical quenching.
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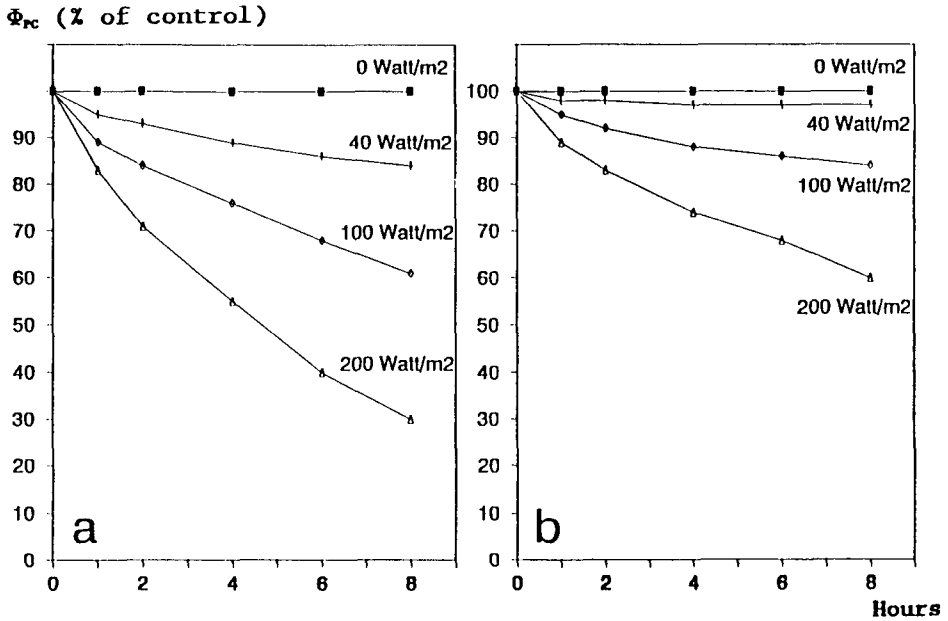

Figure 3 -Average decrease of Φ_{PC} for three maize hybrids as a consequence of photo-inhibition by pretreatment at different light intensities.
The pretreatment temperature of the leaf disks was 5 °C (a) and 17 °C (b)

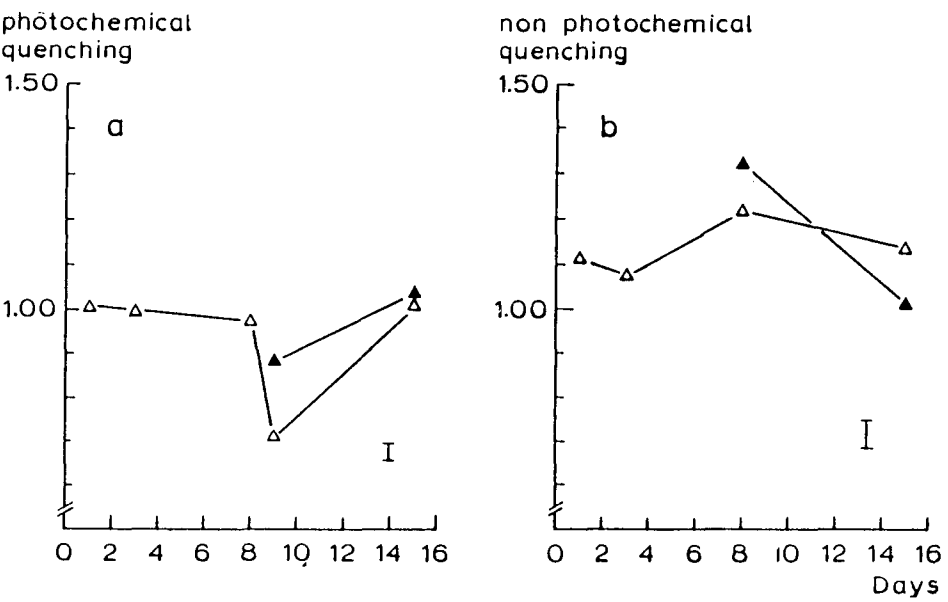

Figure 4 - Time courses of fluorescence parameters during water stress, relative to control treatment, for "old" leaves (open triangles) and "young" leaves (closed triangles), (a) electron transport rate (q_P), (b) energy state of the leaves, (q_N).

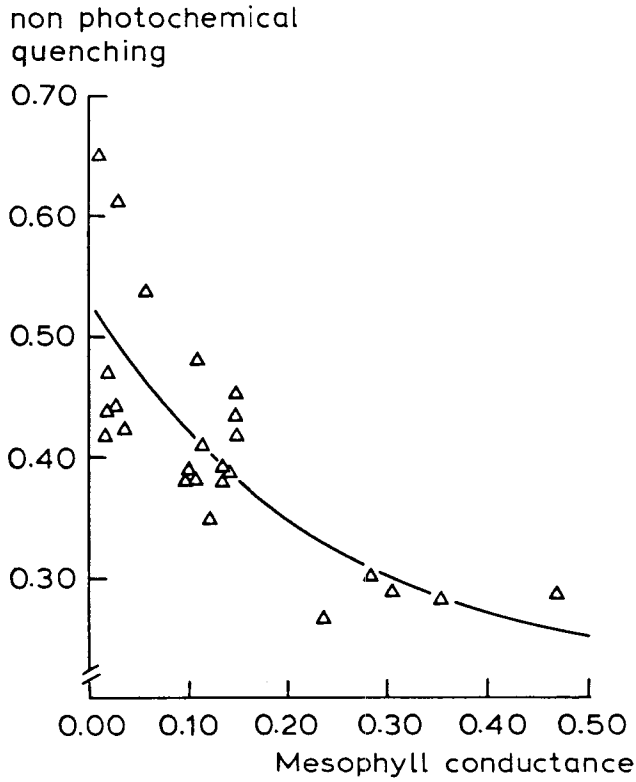

Figure 5 - Relation between mesophyll conductance (cm s^{-1}) and non-photochemical quenching.