

Use of Chlorophyll Fluorescence Methods for the Study of Physiological Condition and Resistance against Abiotic Factors of Grapevine

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Abstract: The purpose of this study was to explore influence of abiotic factors, such as high temperature, water deficiency and high solar radiation on the photomembrane of grapevine leaves. Grapevine leaves were collected from variety Rkatsiteli (*Vitis vinifera*) and placed at a temperature of +45 °C and +55 °C for 5 min, respectively. The relative volume of water in leaves was gradually reduced to 50%, and then leaves were irradiated with 6,000 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ of white light. Changes provoked by stressful abiotic factors were determined using rapid and delayed chlorophyll fluorescence methods. It was shown that value of variable component of chlorophyll fluorescence (F_v), intensity of electron transport between the photosystems (ETR), intensity of expended electrons in carboxylation (ETR_n) and oxygenation (ETR_p) and index of non-photochemical quenching (NPQ), allow studying molecular mechanisms of the impact of abiotic factors and the resulting damage degree. Based on delayed and rapid fluorescence data, it was demonstrated that temperature of +45 °C adversely affects oxygen production system and CO_2 assimilation mechanisms, while at +55 °C, the ETR decreases. Reduction of relative water volume in leaves up to 50%-55% leads to sharp reduction in ETR and inhibition of photosynthesis. In case of irradiation of leaves with high-intensity light of 6,000 $\mu\text{mol}/\text{m}^2\cdot\text{s}$, NPQ of light falling on a leaf increases, thus protecting photosynthesis apparatus from damage.

Key words: *Vitis vinifera*, chlorophyll fluorescence, resistance, abiotic stress.

1. Introduction

Global warming created serious problems for viticulture [1-3]. Processes, such as grapevine resistance against high temperatures, the increased solar radiation and drought, have come to the fore [4-7]. Many scholars developed certain agrotechnical measures for protection of vineyards in conditions of global warming [8, 9].

Considering the above-mentioned, it is obvious that analyzing mechanisms of the impact of abiotic factors on the grapevine, such as high temperature, water deficiency and the increased solar radiation, and the protection of grapevine from the impact acquires particular importance. On the other hand, data indicate that the key process carried out in leaves, i.e., the mechanism of primary processes of photosynthesis,

takes place in chloroplasts, in particular, in the thylakoid membrane [10]. Extreme abiotic factors affect photomembrane and lead to changes in primary processes of photosynthesis, which are reflected in chlorophyll fluorescence properties [11, 12].

The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers [13-15]. Studies in Refs. [16, 17] show that chlorophyll fluorescence intensity in phelloderm of one-year grape shoots is directly proportional to low negative temperature, namely, if temperature decreases, the fluorescence intensity also decreases. Furthermore, the more frost-resistant the vine species is, the faster fluorescence intensity decreases. This result can be used for evaluating relative frost-resistance of various grapevine species.

Chlorophyll fluorescence properties in grapevine

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buds are used for identifying bud damage degree due to frost. Duering et al. [11] show that the ratio of variable fluorescence to the maximum value of fluorescence (F_v/F_m) may be used to determine the frostbitten buds. If the value of the above ratio varies between 0.2 and 0.3, the bud is frostbitten.

Base on the above-mentioned, the objective of this study was to link changes due to abiotic factors (high temperature, water deficiency, increased solar radiation) with chlorophyll fluorescence properties.

2. Materials and Methods

Experiments were carried out on Georgian grapevine variety—Rkatsiteli (*Vitis vinifera*). Sample leaves were collected from vineyards in Vashlidjvari, Georgia, as well as two-year-old greenhouse-grown grafted saplings. First, cut one-year grape shoots with 5-7 leaves and then they were quickly placed in water. After 1 h of adaptation, rapid and delayed fluorescence properties were studied. The shoot was then transferred from water to laboratory table, and the changes in the same properties were measured considering the reduction of water in leaves. Relative reduction of water volume (RWC) in leaves was estimated using Eq. (1):

$$RWC = \frac{M - M_d}{M} \quad (1)$$

where, M = leaf mass; M_d = dry mass, it was determined by holding the leaf in the drier for 24 h at 105 °C.

In order to determine high temperature impact, vine leaves were placed in a basin of water of +45 °C and +55 °C, respectively. In order to mimic high radiation of sunlight, the leaves were irradiated with 6,000 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ of white light in the greenhouse for 10, 20, 30 and 60 min, respectively. Each experiment was conducted for five leaves and was repeated three times.

Activity of the primary processes of photosynthesis apparatus in grape vine leaves was studied with the method of delayed fluorescence. The delayed

fluorescence was recorded with the phosphoroscope, and time between object illumination and fluorescence detection amounted to 1 ms [18]. The rapid fluorescence was evaluated with portable photometric apparatus PAM (H. Walz, Effeltrich, FRG) [19].

3. Results and Discussion

The chlorophyll fluorescence properties are directly linked to the primary processes of photosynthesis, while any changes in the processes can be reflected in changes of fluorescence properties. In the induction curves of delayed fluorescence (Fig. 1), the fast phase O-I-D is linked to the electron transport, the slow phase D-P is linked to energizing of the photomembrane, while the phase P-S is linked to CO_2 assimilation [17, 18]. Considering changes of these indices of fluorescence, the deterioration degree in the primary processes of photosynthesis, such as electron transport, photophosphorylation and CO_2 assimilation, can be judged. It was shown in Ref. [17] that the coefficient $K = (P - S)/S$ is directly linked to the photomembrane activity; the decrease of the photosynthesis activity directly correlates with its decrease.

The variable fluorescence (F_v) of the Rkatsiteli leaves was calculated as Eq. (2):

$$F_v = F_m - F_0 \quad (2)$$

where, F_m is the maximal and F_0 is the minimal fluorescence yield of a dark-adapted sample.

F_v is linked to the photosystem II reaction centers (PS II) activity. In normal conditions, its value equals $F_v/F_m = 0.6-0.7$. The stress impact on leaves results in decrease of its value, according to which the reversibility of the stress-induced damages can be judged. According to Ref. [19], parallel measurements of variable fluorescence and photosynthesis intensity provide for calculation of electron transport intensity between the photosystems (ETR), as well as intensity of expended electrons in carboxylation (ETR_n) and oxygenation (ETR_p) as Eqs. (3)-(5):

$$ETR = Y \times 0.85 \times R \times PAR \quad (3)$$

where, Y is intensity of variable fluorescence, PAR is active radiation of photosynthesis ($\mu\text{mol}/\text{m}^2\cdot\text{s}$) and $R = 0.5$, is a constant.

$$ETR_p = \frac{1}{3} [ETR + 8 \times (A + R)] \quad (4)$$

$$ETR_n = \frac{1}{3} [ETR - 4 \times (A + R)] \quad (5)$$

where, ETR is total electron transport intensity, A is intensity of photosynthesis and $R = 0.5$, is a constant.

Vast part of the intensity of light falling on a leaf is linked to so-called non-photochemical quenching (NPQ). This process is the mechanism that guards a leaf from high intensity of solar irradiation (photoc burn). It is with these processes which the rapid fluorescence NPQ index does connect, and the NPQ could be calculated with Eq. (6):

$$NPQ = \frac{F_m - F_m^t}{F_m^t} \quad (6)$$

where, F_m is the maximum fluorescence of the dark-adapted leaf to the first light impulse, while F_m^t is the maximum value of fluorescence of a leaf

illuminated with constant red light.

The value of the NPQ index is directly connected to the light of non-photochemical quenching [19].

In following, the study tried to use the above properties of chlorophyll fluorescence to explore the impact of abiotic factors of high temperature, water deficiency and high radiation of light on vine leaves.

3.1 Impact of High Temperature on Vine Leaves

Fig. 1 demonstrates induction curves of the Rkatsiteli leaves for 5 min incubation at high temperature. It is evident that at $+45^\circ\text{C}$, the decrease of slow phases D-P and P-S did occur, which depends on the decrease of ΔpH values, as well as on the decrease of CO_2 assimilation velocity. However, in this case, damage is reversible. At $+55^\circ\text{C}$, the processes of photophosphorylation and CO_2 assimilation are completely inhibited, and also the ETR decreases significantly. Irreversible damage to the photomembranes does occur in this case, i.e., once $+55^\circ\text{C}$ temperature stress is relieved, the primary processes of photosynthesis do not return to primary

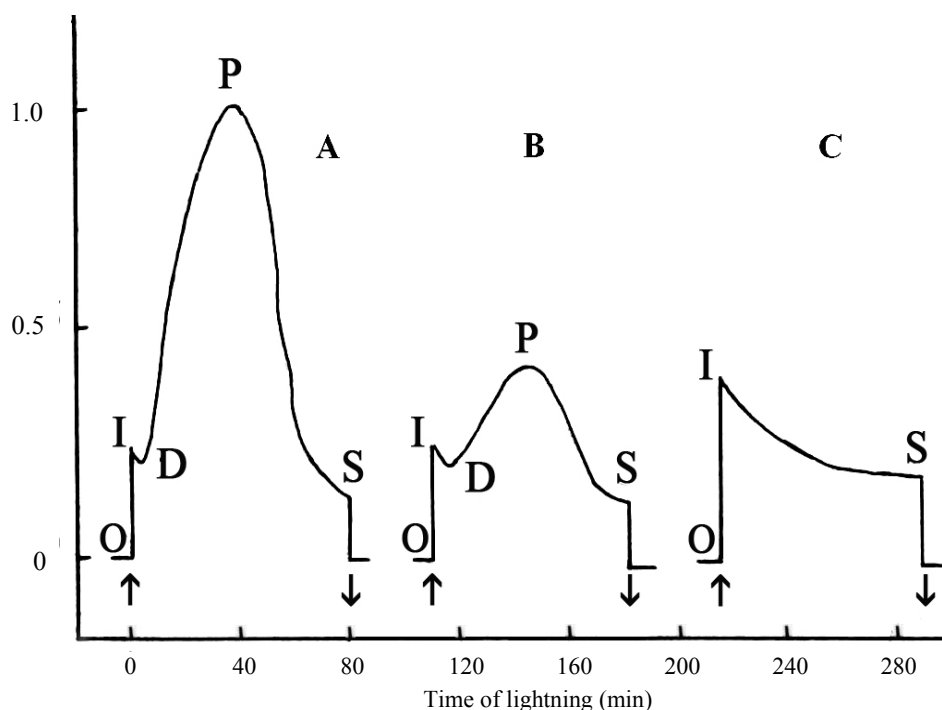


Fig. 1 Induced straight lines of the delayed fluorescence in the Rkatsiteli leaves.

A: control; B: incubated at $+45^\circ\text{C}$; C: incubated at $+55^\circ\text{C}$. Arrows point to the moments of light on and off.

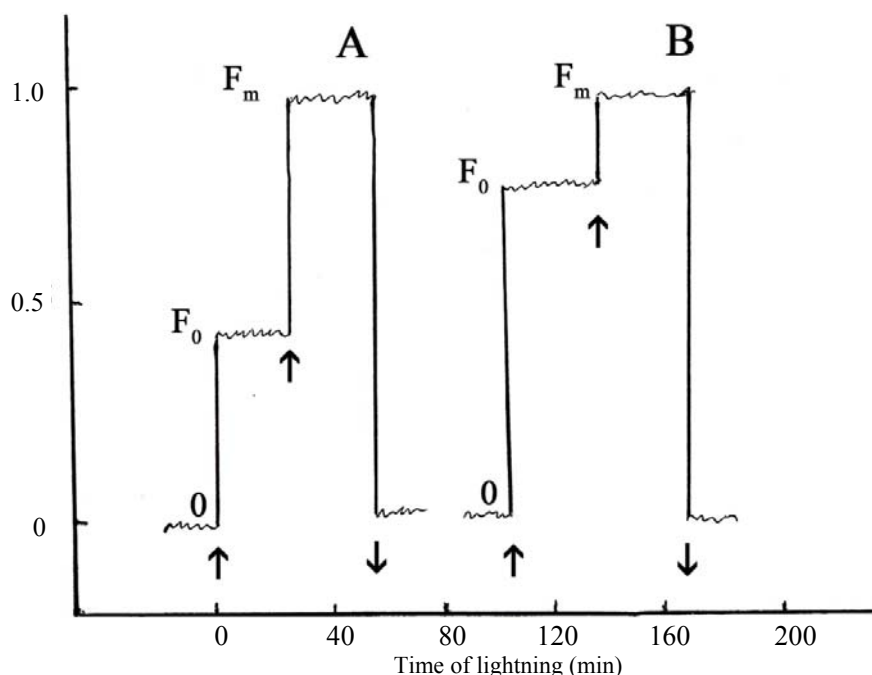


Fig. 2 Induction straight lines of the rapid fluorescence in the Rkatsiteli leaves by water stress.

A: control; B: relative volume of water 60%. Arrows point at the light on and off moments.

values and the leaf is damaged.

3.2 Impact of Water Deficiency on Vine Leaves

In case of water deficiency, the value of variable fluorescence decreases (Fig. 2), indicating the decreased photosynthesis intensity. During the decrease of relative volume of water from 95% to 75%, the *ETR_p*, which is linked to carbonation, decreases sharply. The result obtained shows that from primary processes of photosynthesis in grapevine leaves, CO_2 assimilation is extremely sensitive to dehydration. The process of photophosphorylation decreases as well. However, the latter changes are reversible.

If the relative volume of water in the grapevine decreased up to 50%-55%, the sharp decrease in *ETR* does occur and photosynthesis is inhibited to zero. In such case, the plant undergoes irreversible damage [12].

3.3 Impact of High-Intensity Light on Vine Leaves

In case of high light irradiation ($6,000 \mu\text{mol}/\text{m}^2\cdot\text{s}$ during 10 min), the *ETR* decreases; however, *ETR_n*

increases and the index of *NPQ* increases, which should be due to non-photochemical shutdown of excessive light illumination. From the biochemical point of view, the defense mechanism against high-intensity illumination should be connected to the chlorophyll transformation; at high intensity, illumination violaxanthin transforms into zeaxanthine [20]. However, if vine leaves are exposed to light of $6,000 \mu\text{mol}/\text{m}^2\cdot\text{s}$ intensity for 30 min or 60 min, the defense mechanism against high-intensity illumination will not be able to function and the leaf burn occurs, i.e., the process will become irreversible.

4. Conclusions

The study showed that during high temperature exposure of vine leaves, photophosphorylation and CO_2 assimilation are the first to decrease. Further increase in temperature leads to the reduction of *ETR*. And damage caused by exposure to $+45^\circ\text{C}$ for 5 min is reversible, while damage caused by exposure to $+55^\circ\text{C}$ is irreversible.

It also showed that during irradiation of vine leaves with $6,000 \mu\text{mol}/\text{m}^2\cdot\text{s}$ high-intensity light for 10 min,

the *ETR* decreases. At that point, defense mechanism against high-intensity illumination is activated in the form of non-photochemical shutdown of excessive light illumination. In this case, stress effect is reversible. However, in the event of exposure to the light of the same intensity for 30-60 min, leaf damage becomes irreversible.

CO₂ assimilation is extremely susceptible to water volume reduction in vine leaves, and photophosphorylation decreases as well. It was found that if relative volume of water in leaves reduced to 75%, damage caused by water stress is reversible. However, if water content reduced up to 55%, leaf damage becomes irreversible. In a word, chlorophyll fluorescence properties of vine leaves, including *Fv/Fm*, *ETR*, *ETRn*, *ETR_p* and *NPQ*, allow studying molecular mechanisms of the impact of abiotic factors and the resulting damage degree.

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