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PLANTS AND CULTIVATIONS KNOWLEDGE-BASED DIAGNOSTIC SYSTEM

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Abstract - Parasitic attacks and environmental stresses may decrease the yield of crop plants and even cause damages to forests. A diagnostic system has been developed for monitoring early stress states in plants in order to set up the most suited intervention and keep plants in their optimal state. The study of natural leaf fluorescence gives useful informations about photosynthetic apparatus so that it appears to be a valid inspection tool for monitoring plants metabolism. The instrument integrates the knowledges of human experts and laboratory diagnostic techniques for an on-site and non-intrusive analysis and a neuro-fuzzy data fusion for the overall response about plants health.

Keywords: plant stress, knowledge-based, diagnostics

1. INTRODUCTION

As well as any other living organism, plants are not isolated systems: they grow up and modify their behaviour in relation to what happens around them and inside of them: an environmental change, an unexpected cold or hot period, an attack of a fungus or other parasites, a lack or an excessive quantity of water, a pollutant agent or an abuse of pesticides and many other causes may induce stress conditions in plants. The direct consequence is a loss of vitality and, in relation to crop plants, a loss of yield. The usual procedure is to diagnose the specific problem and apply the most appropriated treatment for drawing back plants in their optimal condition. Unfortunately the correct identification of the characteristics symptoms of a stress is not an easy task even for an expert, especially if it has to be done in an early state when the treatment would be more efficient. The key point is to develop a monitoring system which could reveal the stress states and suggest the most suited intervention.

2. TECHNOLOGIC PROSPECTION

In technical literature many different inspection methods are described. They are based on nuclear magnetic resonance (NMR), protein luminescence and bioluminescence, thermal imaging and fluorescence. Each one can retrieve useful informations on plants metabolism. From an accurate technologic prospection the fluorescence measurements appeared to be the most interesting for several aspects, mainly the quantity and quality of retrieved information.

3. MEASUREMENT SYSTEM

A plant under stress modifies its metabolism, so that among many other consequences there are changes in chlorophyll, mineral and water content and concentration, and other plant photosynthetic functionalities. A green leaf has a specific absorption/emission spectrum due to the contained photosynthetic pigments: if exposed to a UV-radiation a part of the absorbed energy is used for the photosynthetic process while another part is emitted as heat and fluorescence light at longer wavelengths. The typical emission spectrum is shown below.

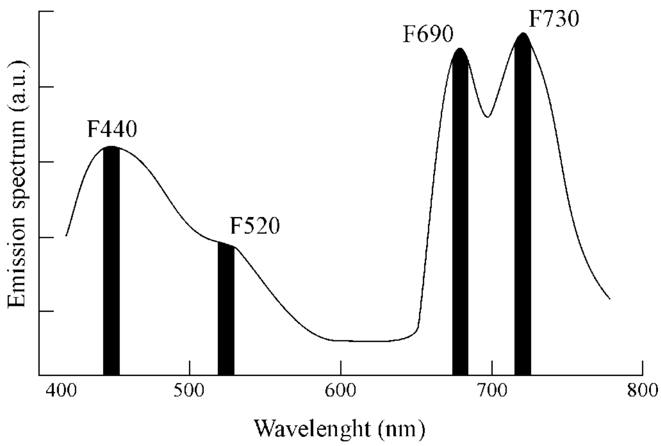

Fig. 1. Typical chlorophyll emission spectrum

The peak in the blue region (440 nm), the shoulder in green (520 nm), the low peak or shoulder in the red (680 nm) and the peak in the far-red (740 nm) are called respectively F440, F520, F690, F740. The instrument uses these values in ratiometric form, in particular it evaluates F440/F520, F440/F690, F440/F740 and F690/F740 ratios. The red and far-red emissions are typical of chlorophyll while the blue and green fluorescences are primarily emitted from cinnamic acids bound to cellulosic walls so that they can be found in non-green tissues such as roots, flowers and, in green leaves, in the chlorophyll free epidermal cells and in the major leaf veins that is where the fluorescence is not reabsorbed by the photosynthetic apparatus. Other ratios related to metabolic parameters can be considered but these have been chosen because they are the most interesting ones, leaving the others as future improvements of the instrument.

Further informations can be taken from the fluorescence dynamic response of F740. The behaviour of photosystem II (a protein responsible of a step of photosynthetic process) is probed by means of the rise kinetics of the chlorophyll in a dark adapted green leaf upon illumination with a saturating actinic light.

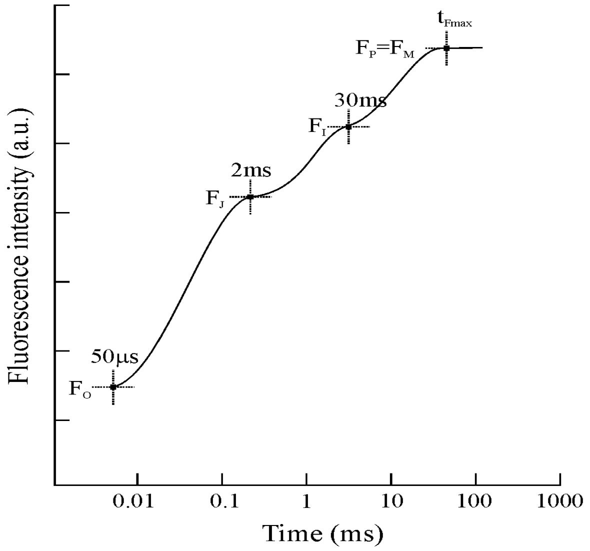

Fig. 2. Typical chlorophyll fluorescence rise.

Four values are sampled from the rise transient: F_O at 50µs from the onset of illumination, F_J at 2ms, F_I at 30ms and $F_P=F_{max}$ at 200ms. An additional value is the initial slope defined as $M_0=V_{300\mu s}$, where $V_{300\mu s}$ is the fluorescence at 300µs normalized with F_O. It has to be noted that these timings are typical values.

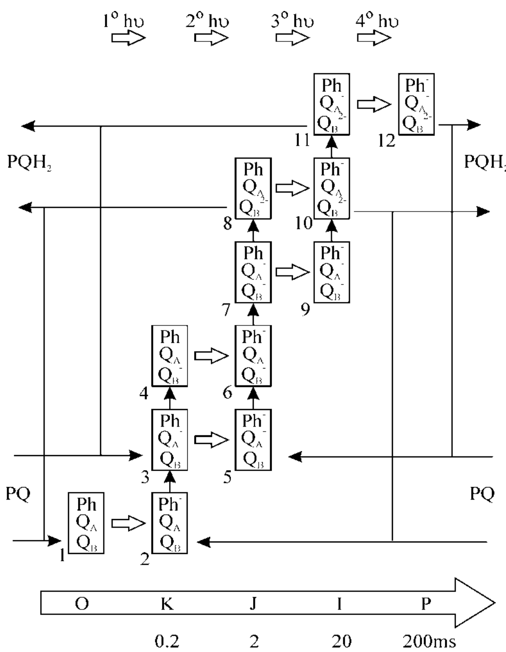

Fig. 3. Fluorescence rise kinetics [3]

The diagram in Fig. 3 shows the correlation between the rise form and the accumulation and transformation by light absorption of plastoquinones Q_A, Q_B, Pheophitine and their reduced forms in the reaction centres. The O-J phase corresponds to a complete reduction of the primary electron transport acceptor Q_A of photosystem II, the J-I phase fluorescence is due to the water splitting activity of the photosystem II donor side and the third phase I-P is related to the oxidised plastoquinone pool.

A laboratory standardized procedure called O-J-I-P test from the names of the sampled values is based on the fluorescence rise transient and permits to calculate parameters related to the photosynthetic apparatus which are reported in the following tables.

Table 1. Specific energy fluxes per reaction centre and per cross section

Specific energy fluxes	Per reaction centre	Per cross section
Absorption	ABS/RC	ABS/CS_M
Trapping	TR_0/RC	TR_0/ CS_M
Dissipation	DI_0/RC	DI_0/ CS_M
Electron transport	ET_0/RC	ET_0/ CS_M

Table 2. Yields and flux ratios

	Yield	Yield as ratio of fluxes
Maximum quantum yield of primary photochemistry	φ_{Po}	φ_{Po}
Quantum yield of electron transport	φ_{Po}	φ_{Po}
Trapped exciton movement into electron transport chain efficiency	ψ_0	ψ_0

Table 3. Indexes and performance parameters

Index	Name	Notes
Structure function index	SFI_{Po}	Combines parameters favouring photosynthesis
	SFI_{No}	Related to dissipation
Performance index	PI_{ABS}	Ratio SFI_{Po}/SF_{Ino} relative to absorption
	PI_{CS}	Ratio SFI_{Po}/SF_{Ino} relative to cross section
Total driving force	DF_{ABS}	Relative to absorption
	DF_{CS}	Relative to cross section

4. MEASURING SYSTEM

A light source is modulated by a square wave with frequency and duty-cycle settable by the user for a greater control of the instrument but measures evidenced that these are not critical parameters so that a duty cycle of 50% and a frequency of 0.5Hz are the typical values used in the experiments. The radiation coming from the leaf is filtered by a set of interferential narrow band filters and then focalised on a PIN photodiode. The signals are pre-conditioned by a dedicated analog front-end. Four analog channels of an acquisition card (National Instruments DAQCard-AI-16E-4) are used to sample the signals. The sample rate may be set by the user as well as the number of scans; a 100ksamples/sec scan rate is used in order to correctly acquire the fluorescence fast rise.

The light source modulation, the acquisition hardware, the elaboration and storage of acquired data are controlled using virtual instrumentation technics via the acquisition card.

The acquired data is shown in form of graph and the user can set the markers to obtain the values for calculating the parameters and indices described in Table 1,2,3, that are analysed by the neuro-fuzzy network to evaluate the health status of the plant.

4. MEASURES

Some experiments revealed that there are different responses both in the fast rise fluorescence and in the slow decay after a relatively long time of illumination. In particular, tests have been done with leaves exposed to low and high temperatures and to chemical agents for few minutes. The timescale is in logarithmic form to put in evidence the fluorescence rise shape, while the amplitude axis is linear and reports the voltage read (V) at the output pins of the pre-conditioning front-end. Five markers have been set to retrieve the values at 40us, 200um, 400um, 20ms and 300ms that are the OJIP test requested points. It is evident from the graphs that the shapes of the fluorescence rise are similar for the inspected leaves, but the absolute values and the ratios of the retrieved values present evident differences that are also reported in the OJIP parameters calculated for this experiments.

Fig.4: Example of different stresses applied to a leaf compared to the reference leaf

In Fig. 5 the fluorescence waveforms relative to four light pulses are shown using a linear timescale so that the different behaviours of the reference leaf fluorescence and the stressed leaves fluorescence appear evident also when analysed on a longer timescale.

A photochemical quenching is revealed in reference leaf and from other measurements (not reported); it has been observed that the maximum fluorescence value decreases to reach a steady state in few minutes depending on the energy absorbed by the leaf during the measure. On the contrary the stressed leaves present no decay.

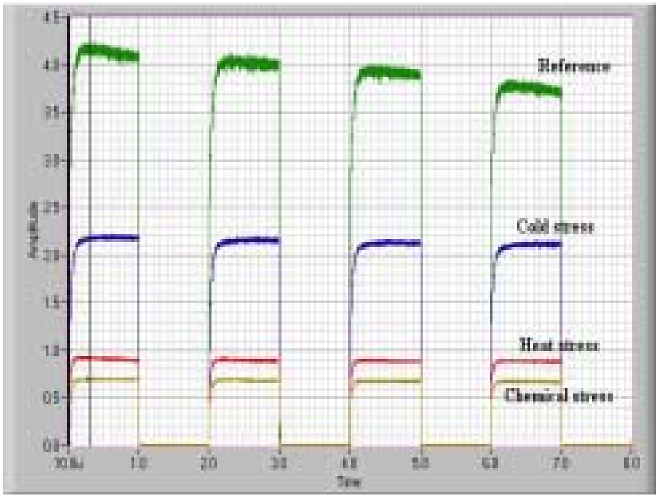

Fig. 5. Example of stressed leaves responses compared to the reference leaf after some light pulses

Measuring the intensity at the specified emission bands it has been noticed that at low concentration of chlorophyll F690 is greater than F740 which appears as a shoulder, instead for greater chlorophyll contents F690 decreases to a local peak or even a shoulder as this wavelength is reabsorbed by chlorophyll-a.

F520 is related with different compounds bound to cell walls. In the mesophyll this emission is reabsorbed by the other wide absorption spectrum pigments for maximising energy conversion in photosystem II. This band is used to reveal the presence/absence of some compounds and pigments but it is also linked with lack or abundance of minerals and water in the plant or its growth conditions.

5. CONCLUSIONS

Parameters setting and instrument calibration is not needed as the instrument retrieves ratiometric measurements from the static method and values normalized to F_0 from the dynamic method. In this way it is intrinsically independent from many environmental and system variables such as superficial leaf characteristics and light stimulus instability. Furthermore given the short measuring time repeated measurements are done to make the instrument insensible to the used electronic components so that no pre-trimming is needed.

With opportune modifications the instrument can perform remote measurements: a plant (or group of plants) integral inspection could be done using both sun light or UV source as illumination, obtaining an overall information about an entire crop.

The advantage of the developed system respect to others and the reasons that lead to the choice of this method lay mainly in the amount and quality of acquired data; it has been observed that each single value is not typical of a specific stress or plant, but carries implicit informations that can be extracted by an accurate data fusion and analysis.

The characteristics of the instrument and the chosen inspection method let the system to be applied to any situation of plants and stresses as data elaboration is demanded to a learning neuro-fuzzy whose importance is evident also in the presented high level response: the user is not supposed to have specific knowledges on the measure method or the biological processes, as they have been transferred from the human experts to the instrument.

The next improvement is to use a camera as the sensible element instead of photodiodes and to introduce imaging analysis so that many more informations about the status of a plant could be retrieved, among which the most important is the fast localization and mapping of stress affected areas within a leaf, a plant or even a crop.

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