OJIP FLUORESCENCE TRANSIENT ANALYSIS – A RAPID AND NON-INVASIVE METHOD TO DETECT GENOTYPIC VARIATIONS IN THE RESPONSE TO SOIL CHARACTERISTICS OF RASPBERRY AND BLACKBERRY CULTIVARS

ANALIZA FAZELOR DE FLUORESCENȚĂ O-J-I-P – METODĂ RAPIDĂ ȘI NON-INVAZIVĂ DE MONITORIZARE ÎN TIMP REAL A RĂSPUNSULUI UNOR GENOTIPURI DE ZMEUR ȘI MUR LA CONDIȚIILE DE CULTURĂ

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Abstract: *The fast O-J-I-P fluorescence transients and their quantification by JIP-test provide a rapid, reliable and non-invasive tool to detect real time changes in the functionality of the photosynthetic apparatus and plant vitality in physiological or stress conditions. In this communication, we have used for the first time the fast Chl a fluorescence transients to assess genotypic variations in the response of two raspberry (Opal and Cayuga) and two blackberry (Thornfree and Lochness) cultivars to soil characteristics. The plants were maintained in the greenhouse at 25±5°C on two different substrates: soil with pH 5.8 and a soil:peat mixture (1:2) with pH 4.7. The kinetics of Chl a fluorescence showed genotypic variations in the magnitudes and rise of O -J, J-I, and I-P phases in response to soil conditions mainly in raspberry. On a soil:peat mixture, Cayuga showed inhibition of the J-I phase of fluorescence and a lower P-step whereas in Opal both O-J and J-I steps were largely inhibited with a highly suppressed P-step. In blackberry, genotypic variations were mainly obtained for I step which was much lower in Thornfree. The Jip-test revealed that the two raspberry cultivars have different respnsivenss to soil pH with Opal being more responsive than Cayuga. Similarly, Thornfree was more responsive to soil pH than Lochness.*

Key words: *chlorophyll fluorescence, JIP test, soil pH, raspberry, blackberry*

Rezumat: *În ultimii ani analiza fluorescenței clorofilei a devenit una dintre cele mai folosite tehnici de detectare în timp real a schimbărilor survenite in structura moleculară și funcţionalitatea aparatului fotosintetic ca răspuns la acţiunea factorilor de mediu biotici sau abiotici. Scopul acestei lucrări este monitorizarea răspunsului unor genotipuri de mur și zmeur la condiţiile de pH*

al solului, prin analiza fazelor de fluorescență O-J-I-P induse în urma excitării centrilor de reacţie foliari cu un puls de lumină roşie cu intensitatea de 3000 µmol m^{-2} s^{-1}. Materialul vegetal folosit a fost reprezentat din 2 soiuri de zmeur (Opal şi Cayuga) şi respectiv, 2 soiuri de mur (Lochness şi Thornfree) care au fost plantate în seră pe două variante de substrat cu pH diferit (4.7 si 5.8). În urma analizei parametrilor derivaţi din valorile fluorescenței clorofilei a la anumiţi timpi s-a constatat că la variantele cultivate pe substrat mai acid există o scădere a eficientei fotosintetice ca urmare a amplificării ratei de disipare a energiei luminoase sub formă de căldură.
Cuvinte cheie: *fluorescența clorofilei, , Test JIP, pH-ul solului, mur, zmeur*

Raspberry and blackberry are two species largely cultured in Northern Hemisphere due to their ability to synthesize health-related compounds. Therefore, many studies have been focused on the effect of growth conditions on berry fruit quality and plant productivity. Soil pH is one of the abiotic factors that may limit the culture areal of these species because they require a slight acidic soil (5.5 – 6.2) with good permeability properties. Inappropriate soil conditions may affect photosynthesis and, consequently, plant growth and productivity as well as fruit quality. Therefore, it is important to use fast and simple methods to test the response of plants to soil in order to detect any situation that could alter the photosynthetic processes. Application of chlorophyll a (Chl a) fluorescence is a simple and non-invasive tool to monitor photosynthesis in vivo and in vitro. In a dark-adapted sample, most excitation energy is consumed by photochemistry, thus lowering fluorescence yield through a process termed photochemical quenching. When PS II centers are in their open states, fluorescence yield is low, whereas when they are blocked, particularly by the reduction of the primary quinone electron acceptor (QA) in photosystem II (PS II), fluorescence yield increases. In addition to photochemical quenching, various regulatory or inhibitory processes can lower fluorescence yield, and these are generally termed non- photochemical quenching. Thus, chlorophyll assays were used as indicators of the efficiency or turnover rate of PS II. The fluorescence rise exhibited during the first second of illumination by dark-adapted plants shows a sequence of steps from the initial (F_0) to the maximal (F_m) fluorescence value. As reported by many authors, these steps labeled O, J, I, P show changes under different environmental conditions such as UV light (Sfichi-Duke et al, 2008), low temperature (Sfakianakis et al., 2006) etc. The JIP-test is a quantification of these transients and provides a constellation of structural and functional parameters characterizing the PSII behavior (Strasser et al., 2000). It has been widely and successfully used for the study of PSII activity in various photosynthetic organisms under

physiological and stress conditions allowing the rapid screening of many samples in field of laboratory experimental conditions. In the present contribution we applied for the first time the OJIP-test to test the response of some raspberry and blackberry cultivars to soil conditions (pH and texture) and also to detect genotypic variations in this response.

MATERIAL AND METHODS

Plant material Raspberry (Opal and Cayuga) and blackberry (Lochness and Thornfree) cultivars have been planted in the greenhouse in 6 L pots in soil (pH 5.8) and a soil:peat mixture (1:2) (pH 4.7). The pots have been maintained for a month at 25±5°C temperature and photo-period of 18 h/6 h. In addition to the ambie ntal light, 40W fluorescent lamps which provided a light intensity of 140 µmols $m^{-2}s^{-1}$ have been used for illumination. The fluorescence measurements were conducted after a month of plant adaptation to growing conditions on fully-expanded leaves.

Chl *a* fluorescence measurements Chl a fluorescence transients of intact leaves were measured by a HandyPea fluorimeter (built by Hansatech Instruments, King's Lynn Norf olk, PE 30 4NE, GB). The transients were induced by a red light (peak at 650 nm) of 3000 µmols $m^{-2}s^{-1}$ provided by an array of six light-emitting diodes; they were recorded for 1 s, starting from 50 ms after the onset of illumination, with 12 bit resolution ; the data acquisition was every 10 ms for the first 2 ms and every 1 ms thereafter (for details see Strasser et al., 1995). The leaves were dark-adapted for 30 min before the fluorescence measurements. The fluorescence signal at 50 µsec after the onset of illumination was considered as Fo (Strasser and Strasser 1995). The J step was the fluorescence measured at 2 ms while the I step was the fluorescence value recorder at 30 ms. The maximal fluorescence intensity denotes the Fm values where all the reaction c enters (RCs) are physiologically closed. The Fo state indicates the physiological state when all the RCs are open. From the values obtained for Fo and Fm the maximum yield pf PSII photochemistry (Fv/Fm) has been calculated.

The JIP-test It was used to calculate several parameters such as; absorbance per reaction centers (ABS/RC), energy trapped in the reaction center (TR o/RC), electron transfer rate per reaction center (ETo/RC), energy dissipation per reaction center (DI o/RC) and the density of active reaction centers (RC/CS) (for details see Strasser and Strasser, 1995).

Statistical analysis For each experimental category, five leaves of each plant were measured. Three independent experiments have been performed. The average fluorescence values and standard deviations were also calculated using Excel.

RESULTS AND DISCUSSION

Figure 1 shows fluorescence induction curves for dark-adapted samples. In raspberry (Fig. 1A) Cayuga showed overlapped transients on both substrates. Opal grown in soil exhibited a higher O-J rise than Cayuga, but the I-P step was

lower. In blackberry, Thornfree shows a lower I-P rise than Lochness on both substrates. However, at lower soil pH, the fluorescence highly decreased in Opal and also in both blackberry cultivars, with Thornfree showing a higher sensitivity than Lochness. (Fig. 1B). The transients obtained for ΔF, expressed as the difference between the fluorescence values measured for soil:peat and those measured for soil showed much clearly genotypic variations in the response to low pH of both species.

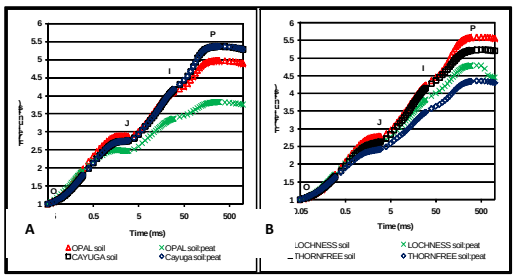

Fig. 1 OJIP transients in raspberry (A) and blackberry (B) grown on soil (pH 5.8) and a

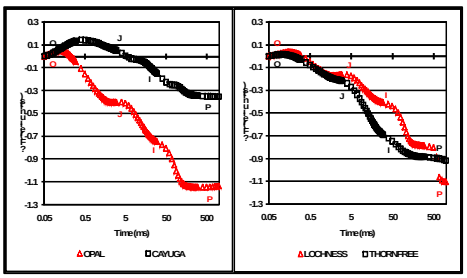

Fig. 2 Genotypic variations in ΔF (calculated as Fsoil:peat pH 4.7 – Fsoil

In raspberry (Fig. 2A), the O-I phase was influenced by genotype and recorded positive values in Cayuga and negative in Opal. This suggests that a lower pH does not affect the electron donation from the oxygen evolving complex in Cayuga as it does in Opal. In spite of the positive O-J rise, the next steps were negative in both raspberry cultivars and this resulted in lower P (corresponding to Fm) peaks. However, the whole fluorescence transient was clearly much inhibited in Opal than in Cayuga. Thus we can assume that Opal is more sensitive to soil pH than Cayuga.

In blackberry, negative values of ΔF and thus Fm were obtained for both cultivars. The O-J phase appears overlapped which suggest that this response is not influenced by genotype (Fig 2B). However, genotypic variations were obtained for I-P step that corresponds to the reduction of quinone molecule acceptors (QA and QB) which was more suppressed in Thornfree than in Lochness. In general, the fluorescence transients recorded on the two soil substrates indicate that both species are sensitive to a lower pH but the degree of sensitivity is influenced by cultivar.

From the fluorescence values several parameters relevant for the estimation of PSII behavior have been calculated using the JIP-test. In raspberry (Fig. 3A), the maximum yield of PSII photochemistry (Fv/Fm) showed higher values in Cayuga than inOpal. It also highly decreased in Opal plants grown at

lower pH. This supports the hypothesis that Opal is more sensitive to soil pH variations than Cayuga.

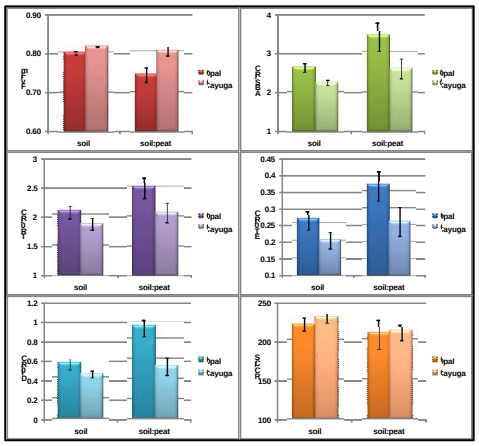

Fig. 3 Genotypic variations of Jip-parameters in raspberry in response to soil characteristics

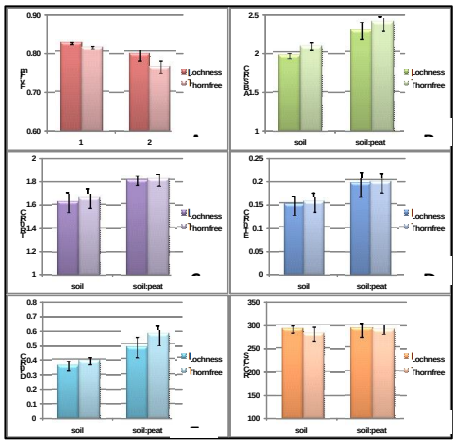

Fig. 4 Genotypic variations of Jip-parameters in blackberry in response to soil characteristics

A similar response was obtained for blackberry where Thornfree showed a higher sensitivity to soil pH than Lochness (Fig. 4A). The decrease in Fv/Fm seems to be related to the increase in antenna size. Absorbance per reaction center (ABS/RC) refers to the photon absorbed by the antenna pigment molecules and it is an indirect measure of antenna size. At lower pH the antenna increased in Opal and Thornfree as compared to Cayuga (Fig. 3B) and Lochness, respectively (Fig. 4B). Beside antenna size, several other parameters also recorded higher values in Opal plants grown at lower pH . These are the energy trapped in the reaction center (Tro/RC) (Fig. 3C) and the electron transport rate (Fig. 3D). It seems that these changes act as a compensatory mechanism to minimize the energetic losses caused by the increase in antenna. Fig. 3D shows clearly that the rate of energy dissipation increased in parallel to antenna in Opal plants grown on a more acidic pH. In spite of this loss, the density of active reaction centers remained more or less constant irrespective of plants growing coditions (Fig. 3F). The above parameters showed similar changes but in a less extent in Cayuga. However, these adaptive responses of Cayuga plants to lower pH impacted in a relatively less significant manner on the density of active reaction centers which recorded similar values to those obtained for Opal (Fig. 3B-F). Thus, Opal plants show a higher responsiveness to pH soil variations than Cayuga and also possess adequate mechanisms to adjust their photosynthetic apparatus in order to keep stable the amount of active reaction centers. In this case, the decrease in Fv/Fm may be an adaptive response to soil pH and not a stress response (Fig. 3A). In

blackberry the genotypic variations in JIP-parameters were less pronounced as those obtained for raspberry. Both cultivars showed lower values of Fv/Fm at pH 4.7 (Fig. 4A) but Thornfree exhibited a higher sensitivity of Fv/Fm to soil pH than Lochness. The decrease in Fv/Fm seems to be the result of an enhancement in energy dissipation (Fig. 4E) due to the increase in antenna (Fig. 4B). Due to the ability of blackberry cultivars to maintain increased trapping (Fig. 4C) and electron transport (Fig. 4D) rates, the density of active reaction centers is not significantly affected by alterations in soil pH (Fig. 4F).

CONCLUSIONS

1. Chlorophyll a fluorescence transients may be used to detect genotypic variations in the response of raspberry and blackberry cultivars to soil pH.
2. Opal and Thornfree showed higher responsiveness to alterations in soil pH than Cayuga and Lochness, respectively.
3. Although all cultivars showed decreased photochemistry at lower pH, the alterations in the JIP-test parameters indicate that these are adaptive (and not stress) responses to soil pH lowering.

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