Photoacoustic detection of ethylene concentration in cherry tomatoes

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In this article, a particular attention is paid to the role of ethylene hormone in cherry tomatoes, from opening flowers to organic mature red fruits. We also assessed the effect of nitrogen flow on cherry tomatoes using a very sensitive laser photoacoustic spectroscopy system. The character of the determinations makes it possible to determine the emission sites of the gaseous plant hormone ethylene and its importance. In aerobic conditions, we monitored the evolution of ethylene concentration from flower to mature red fruit. The nitrogen flow increases the production of ethylene in green and mature cherry tomato fruits and stops the production of ethylene in flowers.

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1. Introduction

The history of the discovery of the ethylene biosynthetic pathway in higher plants represents a good example of advancement of science through the stepwise utilization of novel scientific concepts, plant models and methodologies [1, 2].

The gaseous plant hormone ethylene is an olefin hydrocarbon produced by all plants. Despite its simple chemical structure, it orchestrates a myriad of complex functions. Ethylene controls many diverse metabolic and developmental processes in plants. It has been shown that ethylene is produced from essentially all parts of higher plants, including leaves, stems, roots, flowers, fruits, tubers, and seedlings [2-5].

The biosynthesis of the endogenous hormone ethylene (Fig.1) inside plant tissues depends on the activities of certain enzymes, the rate of outward diffusion and the rate of metabolization; it starts with the conversion of the amino acid methionine into S adenosyl-L-methionine (SAM or AdoMet) by the enzyme Met Adenosyltransferase [6-12].

SAM is converted into 1-aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase (ACS). The activity of ACS is the rate-limiting step in ethylene production; therefore regulation of this enzyme is the key for the ethylene biosynthesis.

The final step requires oxygen and involves the action of the ACC-oxidase enzyme (ACO), formerly known as the Ethylene Forming Enzyme (EFE) [7-13].

Ethylene biosynthesis (Fig.1) can be induced by endogenous or exogenous ethylene. The action of ethylene is not only controlled by endogenous ethylene concentrations in tissues, but also by the tissue sensitivity. It is widely assumed that molecules involved in ethylene perception and in the transduction of the signal probably controls how much ethylene is required to evoke a physiological response [14].

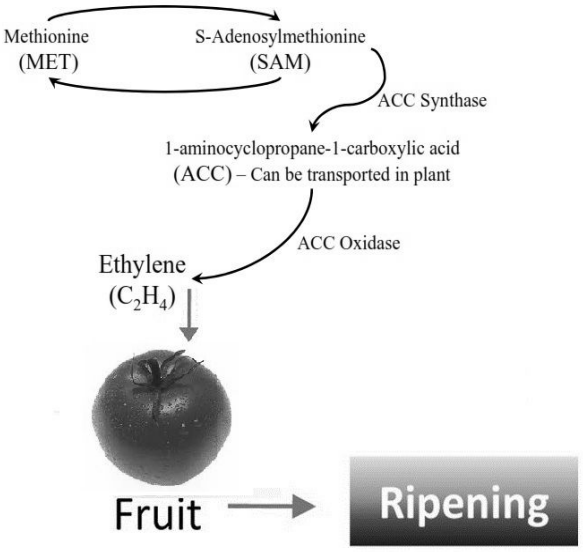

Fig. 1. The ethylene biosynthetic pathway.

Environmental clues can induce the biosynthesis of the plant hormone. Flooding, drought, chilling, wounding, and pathogen attack can induce ethylene formation in the plant.

In flooding, root suffers from lack of oxygen, or anoxia, which leads to the synthesis of 1-Aminocyclopropane-1-carboxylic acid (ACC). ACC is transported upwards in the plant and then oxidized in leaves. The product, the ethylene, causes the epinasty of the leaves.

In plants, ethylene stimulates leaf and flower senescence; stimulates senescence of mature xylem cells in preparation for plant use; inhibits shoot growth except in some habitually flooded plants like rice; induces leaf abscission; induces seed germination; induces root hair growth, thus increasing the efficiency of water and mineral absorption; induces the growth of random roots during flooding; stimulates epinasty – leaf petiole grows out; influences leaf hangs down and curls into itself; stimulates fruit ripening; induces a climacteric rise in respiration in some fruit which causes a release of additional ethylene; affects neighboring individuals; increases disease/wounding resistance; triple response when applied to seedlings – stem elongation slows, the stem thickens, and curvature causes the stem to start growing horizontally; inhibits stem growth outside of seedling stage; stimulates stem and cell broadening and lateral branch growth also outside of seedling stage.

Synthesis of ethylene is stimulated by auxin and maybe cytokinin as well, while ethylene levels are decreased by light. The flooding of roots stimulates the production of ACC, which travels through the xylem to the stem and leaves, where it is converted to the gas. Also, it interferes with auxin transport (with high auxin concentrations) and inhibits stomatal closing except in some water plants or habitually flooded ones, such as some rice varieties, where the opposite occurs (conserving CO_2 and O_2). Where ethylene induces stomatal closing, it also induces stem elongation. Finally, ethylene induces flowering in pineapples [13-18].

The aim of this study was to determine the ethylene emission in cherry tomatoes (from flower to mature fruit), employing a real time ethylene gas analysis sensitive system.

2. Materials and method

The emitted ethylene concentration measurement is analyzed by laser photoacoustic spectroscopy (LPAS) technique, as it offers a high sensitivity that makes possible to evaluate absorption coefficients on the order of 10^{-8} cm^{-1} [19].

A schematic resonant LPAS setup for photoacoustic detection of ethylene at trace level is shown in Fig. 2.

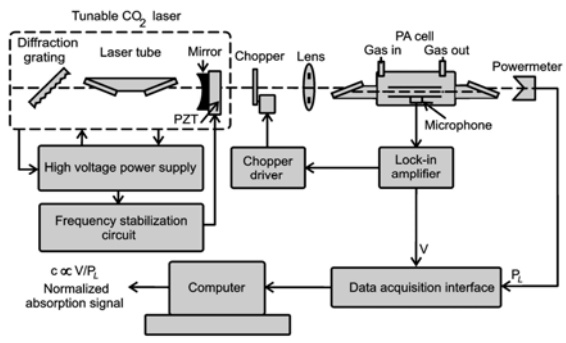

Fig. 2. Typical LPAS system for ethylene gas determination.

The continuous wave laser radiation is amplitude-modulated by a mechanical chopper operating at an acoustic resonance frequency of the photoacoustic (PA) cell. It is then focused by a lens and directed through the resonant cell. The transmitted laser power is monitored with a powermeter -P_L. Inside the cell the radiation produces pressure modulation recorded by microphone as an acoustical signal -V, which is processed by a lock-in amplifier locked to the chopper frequency. The trace gas concentration can then be deduced as being proportional to V/P_L ratio [20, 21].

The power reading after beam passage through the resonant cell can only be used for "transparent" gas samples.

An advantage of LPAS (as a tool for trace gas analysis) is that very few photons are absorbed as the laser beam passes through the sample cell. As a result, notwithstanding the losses from absorption in the windows, the transmitted beam typically has sufficient power for analyzing samples in successive cells, via a multiplexing arrangement. A multiplexed photoacoustic sensor can be used to monitor many different samples simultaneously so that one instrument can be deployed to monitor up to 20 different locations within a clean room, industrial plant or other facility [22].

Following the terminology introduced by Miklos et al. [23], the name 'PA resonator' will be used for the cavity in which the resonant amplification of the PA signal takes place. The term PA cell is reserved for the entire acoustic unit, including the resonator, acoustic baffles and filters, windows, gas inlets and outlets, and miniature microphones (Knowles electret EK-3033 or EK- 23024 connected in series). Finally, PA instrument (PA sensor) stands for a complete setup, including the PA cell, light source (a cw frequency stabilized and line-tunable CO_2 laser tuned on the 10P (14) emission line at 10.53 μm), gas handling system, and electronics used for signal processing.

We used a dual-phase, digital lock-in amplifier Stanford Research Systems model SR 830 with the following characteristics: full scale sensitivity, 2 nV – 1 V; input noise, 6 nV (rms)/ √Hz at 1 kHz; dynamic reserve, greater than 100 dB; frequency range, 1 mHz –102 kHz; time constants, 10 μs – 30 s, or up to 30000 s.

PA cell has a total volume of approximately 1.0 dm^3 and is made of stainless steel and Teflon to reduce the outgassing problems. The PA cell consists of an acoustic resonator tube, windows, gas inlets and outlets, and microphones and it contains an acoustic filter to suppress the window noise. The ZnSe windows are positioned at Brewster angle to their mounts. The resonant conditions are obtained as longitudinal standing waves in an open tube (excited in its first longitudinal mode). To achieve a larger signal, we chose a long absorption path with a length of 300 mm and an inner diameter of the pipe of 7 mm. The fundamental longitudinal wave, therefore, has a nominal wavelength of 600 mm and a resonance frequency of 564 Hz.

The two buffer volumes placed near the Brewster windows have a length of 75 mm and a diameter of 57

mm. The inner wall of the stainless steel resonator tube is highly polished. It is centered inside the outer stainless steel tube with Teflon spacers. A massive spacer is positioned at one end to prevent bypassing of gas in the flow system; the other is partially open to avoid the formation of closed volumes. Gas is admitted and exhausted through two ports located near the ends of the resonator tube. The perturbation of the acoustic resonator amplitude by the gas flow noise is thus minimized. The acoustic waves generated in the PA cell are detected by four Knowles electret miniature microphones in series, mounted flush with the wall. They are situated at the loops of the standing wave pattern, at an angle of 90^o to one another. The electrical output from these microphones is summed and the signal is selectively amplified by the lock-in amplifier [19].

We used a modular software architecture (Keithley TestPoint software) aimed at controlling the experiments, collecting data, and preprocessing information. It helps to automate the process of collecting and processing the experimental results. The software transfers powermeter readings, normalizes data, and automatically stores files. It allows the user to record parameters such as the PA cell responsivity (a constant used to normalize raw data), gas absorption coefficient, number of averaged samples at every measurement point, sample acquisition rate, and the total number of measurement points. This software interfaces the lock-in amplifier, the chopper, the laser powermeter and the gas flowmeter. It allows the user to set or read input data and instantaneous values for the PA voltage, average laser power after chopper, and trace gas concentration [19, 24].

Of great significance in these determinations is the gas handling system due to its role in ensuring gas purity in the cell. It can be used to pump out the cell, to introduce the sample gas in the cell at a controlled flow rate, and monitor the total and partial pressures of gas mixtures. Also, the gas handling system can perform several functions without requiring any disconnections.

To increase the sensitivity and selectivity of the technique, we took some supplementary measures, such as a small glass cuvette for preserving the gas sample, or traps filled with potassium hydroxide (KOH) for retention of the CO_2 and water vapors [25, 26].

Regardless of the glass cuvette chosen (Fig. 3), the system of sample collection must be carefully checked to ensure that there is no gas loss (leakage, decomposition, adsorption to the sample cuvette), or generation of ethylene as a result of chemical reactions within the cuvette. If the sample cuvettes are to be reused, it is equally important to ensure that the cuvettes do not have an ethylene memory (release of ethylene adsorbed onto the inner lining into subsequently collected samples). Possible accumulation of ethylene was prevented by washing the glass cuvette with nitrogen (it is a pure clean non expanding inert gas) at atmospheric pressure for few minutes.

Fig. 3. The device used to contain fruits for the prelevation of the ethylene gas samples.

More details about the PA cell and the experimental protocol are found in other publications [19-26].

3. Ethylene emission from cherry tomatoes flowers to mature cherry tomatoes fruits

Cherry tomato fruits were obtained from local producers (Magurele-Romania, near laboratory), so the fruits have been transported to the laboratory and analyzed immediately after collection. The cultivation area was treated with natural fertilizers and the weeds were controlled with help of hand weeding. The measurements were carried out with intact flowers (weight about 1.4 g, ≈ 9-10 flowers) and fruits (cherry tomatoes with stem) with weight about 33 g (≈ 3-4 cherry tomatoes in a sample) and a diameter per fruit of about 28 mm. The flowers and fruits were introduced into a small glass cuvette with volume of 150 cm^3, connected to the resonant cell and then analyzed at room temperature. As indicated, the cherry tomatoes were investigated in flowers, green and red stages.

The following important parameters were used throughout the experiments for the detection of ethylene gas:

- The power of the laser beam at 10P(14) CO_2 laser line: 4 W;
- Cuvette pressure: ≈ 1024 mbar;
- Responsitivity of the cell: 440 cmV/W;
- Synthetic air: Linde Gaz Romania, 20% oxygen and 80% nitrogen (impurities: hydrocarbons max. 0.1 ppmV, nitrogen oxides max. 0.1 ppmV);
- Nitrogen: Linde Gaz Romania, nitrogen 5.0 (purity 99.999%) and 6.0 (purity 99.9999%);
- Working CO_2 laser line: 10P(14) - λ= 949.479 cm^{-1}, where we have a maximum absorption coefficient for ethylene α= 30.4 $cm^{-1}atm^{-1}$;
- Operating temperature: 23 - 25^0C;
- Glass cuvette total volume: ≈150 mL;
- PA cell total volume: ≈1000 mL;

- Cherry tomatoes samples analysis time: ≈ 3 minutes.

The first objective of the investigation was to detect the ethylene gas from opening cherry tomatoes flowers to organic mature red fruits using LPAS technique in aerobic conditions (Fig. 4).

Over a 20 minutes period we achieved a real-time measurements of ethylene emission produced by 1.4 g cherry tomato flowers and by 33 grams cherry tomato fruits (green and red).

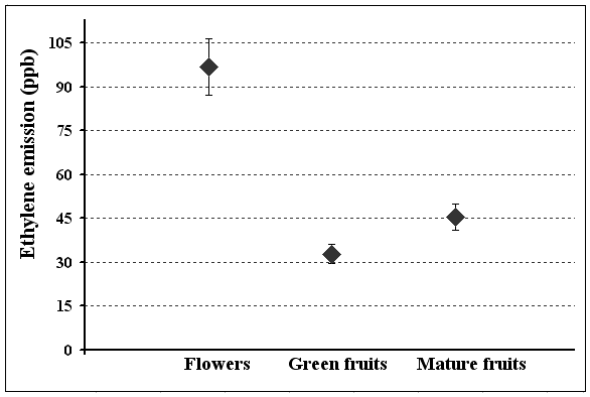

Fig. 4. Ethylene production in cherry tomatoes in aerobic conditions; results for gas detection represent ± 10% of three replicates.

The emission of ethylene in aerobic conditions (with the sample flushed with synthetic air flow at atmospheric pressure - 1024 mbar) for flowers was approximately 97 ppb, for green fruits about 33 ppb, while for red fruits about 46 ppb.

The second objective was to detect the ethylene from opening cherry tomato flowers to organic mature red fruits using LPAS technique in anaerobic conditions (Fig. 5).

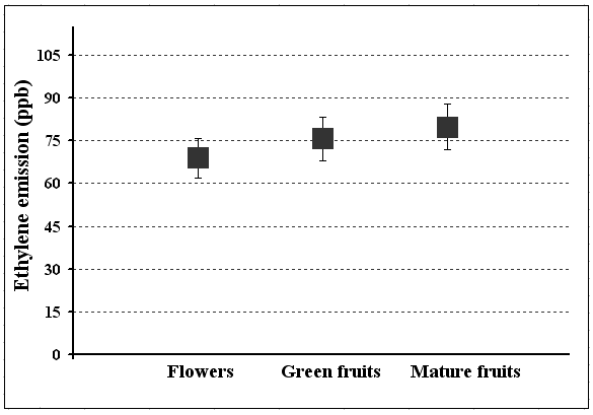

Fig. 5. Ethylene production at cherry tomatoes in anaerobic conditions; results for gas detection represent ± 10% of three replicates.

We replaced the synthetic air flow with the nitrogen flow and we analyzed the evolution of ethylene in anaerobic conditions about 20 minutes.

The emission of ethylene in anaerobic conditions (where the sample was flushed with nitrogen flow at atmospheric pressure) for flowers was approximately 69 ppb, for green fruits about 76 ppb, while for red fruits about 80 ppb.

In synthetic air flow we found that a higher level of ethylene is encountered at flower stage of cherry tomatoes.

The anaerobic conditions increase the production of ethylene gas only at green and red mature cherry tomatoes, while at flower stage they reduce the production of ethylene.

6. Conclusions

In this research we have focused the attention on ethylene evolution in cherry tomatoes flowers and ripening cherry tomato fruits to investigate the major ethylene gas diffusion routes. Using the flow-through the sample gas cuvette coupled with LPAS system, the total ethylene gas emission was observed from the flower stage to the green and red fruit stage (Figs. 4, 5).

The continuous flow system of the LPAS setup allows switching from synthetic air to nitrogen flow at atmospheric pressure. Interchanging aerobic and anaerobic conditions greatly influenced the ethylene emission pattern.

The measurements were performed on intact fresh cherry tomatoes with stem using a cw frequency stabilized and line-tunable CO_2 laser driven detection instrument.

Because are climacteric fruits, cherry tomatoes are characterized by an increase in ethylene biosynthesis and has profoundly varying effects depending on the stage of development (a higher level of ethylene was found at flower stage).

The requirements for LPAS are various and the development and implementation of versatile analytical tools is challenging. Important advantages are: multicomponent capability, sensitivity and selectivity, accuracy and precision, large dynamic range, none or only minor sample preparation, good temporal resolution, ease of use, versatility, reliability, robustness, and a relative low cost per unit.

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