



# Validation of the newly developed *PlantECG* fluorometer against three established chlorophyll fluorescence devices under progressive drought stress in pepper, tomato, and strawberry plants

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## Abstract

Comparing chlorophyll fluorescence data across different fluorometers remains challenging. We compared the new *PlantECG* fluorometer with *PocketPEA*, *HandyPEA*, and *FluorPen* in pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), and strawberry (*Fragaria × ananassa* Duch.) under progressive drought (0–96 h without irrigation). The maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ) measured by *PlantECG* correlated strongly with all reference devices ( $R^2 = 0.72–0.98$ ), although *PlantECG* slightly overestimated  $F_v/F_m$  under severe stress. The performance index ( $PI_{ABS}$ ) showed greater inter-device divergence, with strong correlations in pepper and strawberry ( $R^2 = 0.81–0.89$ ) but weaker ones in tomato ( $R^2 = 0.59–0.67$ ). Absolute  $PI_{ABS}$  values differed substantially: *PlantECG* values were closest to *PocketPEA*, whereas *HandyPEA* recorded 2–5-fold lower values. Correlation quality depended on the species-specific rate of drought response. *PlantECG* reliably tracks drought-induced changes in photosynthetic performance, but species-specific calibration is required before comparing its absolute  $PI_{ABS}$  values across instruments.

**Keywords:** chlorophyll fluorescence; drought stress; fluorometer comparison; *FluorPen*; *HandyPEA*; photosystem II; *PlantECG*; *PocketPEA*.

## Introduction

Drought is one of the most severe abiotic stress factors limiting the growth, development, and productivity of crop

plants worldwide. In the face of ongoing climate change, increasingly frequent and intense drought episodes pose a growing threat to global food security (Lisonbee *et al.* 2025).

## Highlights

- New *PlantECG* fluorometer was tested against three established devices under drought
- Maximum quantum yield of photosystem II correlated strongly between all devices
- Performance index needs species-specific calibration before comparing devices

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**Abbreviations:** ChlF – chlorophyll *a* fluorescence;  $F_0$  – minimum fluorescence (dark-adapted state); the baseline emission when all PSII reaction centres are fully oxidized;  $F_m$  – maximum fluorescence (dark-adapted state); the peak fluorescence emission when the primary electron acceptor  $Q_A$  is transiently and fully reduced by a saturating light flash;  $F_v$  – variable fluorescence ( $F_v = F_m - F_0$ );  $F_v/F_m$  – maximum quantum efficiency of PSII photochemistry; OJIP – polyphasic chlorophyll *a* fluorescence transient (O, J, I, P steps); PAR – photosynthetically active radiation; *PEA* – Plant Efficiency Analyser;  $PI_{ABS}$  – performance index on absorption basis; an integrative parameter combining the density of reaction centres, maximum quantum yield, and the probability of electron transport.

**Conflict of interest:** The authors declare that they have no conflict of interest. *PlantECG* is an invention developed by plant physiologist Prof. Hazem M. Kalaji, together with MSc Ryszard Grodowski, an electronics and IT specialist from the private sector.

Photosynthesis is an exceptionally drought-sensitive process, and its inhibition occurs even under moderate water deficit. Restricted water availability reduces the efficiency of both the light-dependent reactions and the Calvin cycle (Hájek *et al.* 2026). When the amount of absorbed light exceeds the assimilatory capacity of the chloroplasts, damage occurs to the reaction centre of PSII, which constitutes a key element of the electron transport chain (Kalaji *et al.* 2017, 2018). Changes in PSII functioning are widely used as indicators of plant photosynthetic status and can be precisely measured using chlorophyll fluorescence methods (Strasser *et al.* 2004).

Chlorophyll (Chl) *a* fluorescence measurement is a noninvasive, rapid, and highly sensitive method for assessing the functionality of the photosynthetic apparatus in plants under stress conditions and in controlled laboratory environments (Kalaji *et al.* 2017, Chang *et al.* 2025). The method is based on the phenomenon of light emission by chlorophyll molecules following the absorption of light quanta; part of the absorbed energy is re-emitted as fluorescence, and changes in its intensity reflect changes in the efficiency of photochemical processes (Stirbet and Govindjee 2011). Among the numerous fluorescence parameters, the maximum quantum efficiency of PSII photochemistry ( $F_v/F_m$ ), calculated as the ratio of variable fluorescence ( $F_v$ ) to maximum fluorescence ( $F_m$ ) measured after dark-adapting the leaf, is the most widely used indicator of PSII efficiency (Maxwell and Johnson 2000). Values of this parameter close to 0.83–0.85 indicate an optimal state of the photosynthetic apparatus, while their reduction points to photoinhibitory stress or reaction centre damage/inactivity. A second important parameter is the performance index ( $PI_{ABS}$ ), which integrates information on light absorption, efficiency of excitation energy trapping, and efficiency of light energy conversion. This parameter is considered a more sensitive stress indicator than  $F_v/F_m$  (Strasser *et al.* 2010, Ceppi *et al.* 2012, Kalaji *et al.* 2017).

The rapid technological development in the past two decades has led to the emergence of a range of portable fluorometers on the market, enabling noninvasive chlorophyll fluorescence measurements to be taken directly in the field or under greenhouse conditions, without the need for complex laboratory equipment (Kalaji *et al.* 2017). Among the most widely used portable devices are *PEA* (Plant Efficiency Analyser) fluorometers manufactured by *Hansatech Instruments Ltd.* (United Kingdom), including the *HandyPEA* and *PocketPEA* models, which have found broad application in plant physiological research (Strasser *et al.* 2000, Kalaji *et al.* 2017). An alternative to *PEA* devices is the *FluorPen FP 100* fluorometer (*Photon Systems Instruments*, Czech Republic). This is a compact instrument enabling rapid measurement of  $F_v/F_m$  without the use of external dark-adaptation clips. Owing to its ease of use and low cost, *FluorPen FP 100* has also found application in large-scale monitoring of plant condition. However, its diagnostic capabilities are more limited compared to fluorometers capable of detailed OJIP kinetics analysis.

Recently, a new fluorometer of this type, *PlantECG*, was developed by Prof. Hazem Kalaji. This innovative, portable device is designed for easy, precise, rapid, and user-friendly measurements, tailoring it not only to researchers but also to farmers, commercial growers, and students across various educational levels: [https://drive.google.com/file/d/1WQQnZWpq827TI\\_\\_7\\_NO90EDzAAHgSmuE/view](https://drive.google.com/file/d/1WQQnZWpq827TI__7_NO90EDzAAHgSmuE/view).

As a newly introduced technology on the market, *PlantECG* presents a timely opportunity for rigorous evaluation against established, industry-standard reference fluorometers. Conducting this detailed validation will bridge the baseline knowledge gap regarding its comparative performance, providing the foundational benchmarking data necessary to accelerate its adoption across scientific research, education, and precision agriculture (Fig. 1).

*PlantECG* is designed to combine the simplicity and rapid screening capability of compact devices such as *PocketPEA* and *FluorPen FP 100* with the broader analytical functionality of advanced OJIP fluorometers such as *HandyPEA*. Like the *HandyPEA* and *PocketPEA*, *PlantECG* performs fast chlorophyll fluorescence induction measurements based on detailed OJIP kinetics and calculates dozens of key photosynthetic parameters, including  $F_0$ ,  $F_m$ ,  $F_v/F_m$ ,  $PI_{ABS}$ , and other JIP-test indices related to PSII performance. However, unlike the *FluorPen FP 100*, which mainly measures fluorescence values at a few points along the chlorophyll fluorescence induction curve and calculates only a handful of parameters, *PlantECG* enables full, advanced OJIP-based analysis with detailed recording of the fluorescence transient. It measures up to 200,000 points per second, so a measurement is taken every 5  $\mu$ s. *PlantECG* also differs from the other three tested fluorometers in that it integrates additional built-in environmental sensors for PAR, RGB, NIR, temperature, humidity, and atmospheric pressure, allowing simultaneous acquisition of physiological and microclimatic data during measurements. The device is considerably smaller and lighter (95  $\times$  58  $\times$  23 mm; 80 g) than *HandyPEA* and *PocketPEA*, while maintaining high excitation intensity [ $\sim$ 4,000  $\mu$ mol(photon)  $m^{-2} s^{-1}$ ] and



Fig. 1. A newly developed *PlantECG* fluorometer, invented by Prof. Hazem M. Kalaji (<https://plantecg.pl/index.html>).

rapid one-second measurements. In contrast to *HandyPEA*, which is oriented mainly toward advanced research applications with extended protocol customisation and large-scale data handling, *PlantECG* additionally offers unlimited wireless Wi-Fi data transfer to any device (mobile phone, tablet, notebook, or PC) and a simplified graphical display of OJIP-derived physiological status directly on the screen, making it suitable for use by both researchers and non-specialist users. Compared with other fluorimeters, *PlantECG* introduces the novel "Plant ECG" concept, providing a visual stress indicator that is displayed immediately on the screen upon completion of the measurement. This plant physiological status indicator uses user-defined thresholds to classify the sample's conditions as good, moderate, or poor, facilitating direct assessment of plant health, particularly by users without specialised expertise (farmers, growers, and students). Moreover, it offers the possibility of estimating  $F_v/F_m$  without full dark adaptation using the "Kalaji Factor" (see the text below for an explanation of this newly developed Kalaji's factor). Overall, *PlantECG* represents a new generation of compact chlorophyll fluorimeters, combining advanced fluorescence diagnostics with user-friendly operation and integrated environmental monitoring. Further details are provided in the following table.

### Kalaji Factor

Sample dark adaptation represents a critical and often underestimated methodological challenge in chlorophyll fluorescence studies. Usually, there are a few common ways to apply sample dark adaptation before starting chlorophyll fluorescence measurements (to fully oxidise reaction centres and quench any residual fluorescence): attaching dark leaf clips to the leaves for 15–30 min; bringing the plants into a dark room for the same duration; detaching leaves and quickly transporting them to the lab, where they are left in darkness for a comparable period; or performing measurements directly in the field at night.

Recent field-based work by [Rusinowski \*et al.\* \(2026\)](#) provides a cautionary counterpoint to the trend toward eliminating dark adaptation in ChlF measurements. In a comparison of eight dark-acclimation and sample-handling workflows in two field-grown species (*Sida hermaphrodita* and *Miscanthus × giganteus*) – including daytime clip acclimation, evening/night measurements with and without clips, flashlight-assisted night measurements, and detached-shoot measurements after laboratory light exposure – the authors found that OJIP trajectories, including  $F_0$ , the O–J and I–P phase kinetics, the area above the transient, and  $F_v/F_m$ , varied significantly depending on workflow, and that these effects were species-specific in both magnitude and direction ([Rusinowski \*et al.\* 2026](#)). Critically, short daytime clip acclimation and prolonged natural night-time darkness did not produce equivalent reference physiological states, indicating that "dark adaptation" is not a single, standardised condition but is instead sensitive to timing, duration, prior light

exposure, and sample handling. This finding underscores that the question of how (and whether) to dark-adapt samples for OJIP/ChlF measurements remains an open methodological issue: rather than being a solved problem in favour of either strict dark adaptation or its omission, workflow choice must be deliberately selected for the biological question at hand, held constant within a given comparison, and fully reported ([Rusinowski \*et al.\* 2026](#)).

Although the standard chlorophyll fluorescence (ChlF) measurement protocol, including the  $F_v/F_m$  parameter, traditionally requires 15–30 min of dark adaptation to fully oxidise PSII reaction centres, a growing body of methodological research indicates that measurements without dark adaptation can provide meaningful physiological information, particularly for field applications, high-throughput phenotyping, and continuous monitoring ([Murchie and Lawson 2013](#), [Kalaji \*et al.\* 2014, 2017](#)). [Schansker \*et al.\* \(2006\)](#) showed that analysis of OJIP transient recovery kinetics after illumination yields reliable data even without full dark adaptation, and [Bussotti \*et al.\* \(2020\)](#) emphasised that prompt chlorophyll fluorescence (OJIP) is particularly well-suited for large-scale ecological surveys, where obtaining a large number of measurements quickly is often more valuable than achieving the fully relaxed dark-adapted state required by strict protocols – making rapid, ambient-condition measurements the more practical choice. Similarly, a study on *Coffea canephora* ([de Souza \*et al.\* 2026](#)) demonstrated that the dark-adaptation step could be omitted when the entire sample preparation procedure was carried out in darkness, effectively ensuring an equivalent physiological leaf state. Direct experimental evidence for the feasibility of measuring ChlF without dark adaptation is also provided by more recent studies. [Noble \*et al.\* \(2017\)](#) demonstrated *in vivo* that changes in chlorophyll fluorescence lifetime can serve as a noninvasive, spatially resolved readout of PS II-inhibiting herbicide activity, with all measurements – cellular-level (multiphoton FLIM), single-point (fibre-optic probe), and macroscopic (wide-field FLIM) – performed on non-dark-adapted plants. [Xia \*et al.\* \(2023\)](#) went a step further by developing a machine-learning model (LSSVM) that predicts the dark-adapted  $F_v/F_m$  value from ChlF signals measured without dark adaptation, achieving high prediction accuracy (correlation coefficients up to 0.974) across a dataset of over 7,000 samples from six plant species under diverse environmental conditions. Independently, [Tietz \*et al.\* \(2017\)](#) proposed the NPQ<sub>(T)</sub> parameter, which allows estimation of nonphotochemical quenching (NPQ) without requiring an  $F_m$  measurement from a fully dark-adapted leaf, instead using the steady-state  $F_0'$  value, substantially reducing measurement time and eliminating artefacts caused by leaf or chloroplast movement.

Taken together, these studies – spanning fluorescence-lifetime-based approaches ([Noble \*et al.\* 2017](#)), machine learning ([Xia \*et al.\* 2023](#)), and reformulation of the NPQ equation ([Tietz \*et al.\* 2017](#)) – provide a consistent body of evidence that key chlorophyll fluorescence parameters,

| Feature                            | <i>PlantECG</i>                                                                                                                                                                                                                                                                                                                                                                           | <i>HandyPEA</i>                                                                                                               | <i>PocketPEA</i>                                                                                                              | <i>FluorPen FP 100</i>                                                                                                        |
|------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| Device type                        | Portable chlorophyll fluorometer with integrated environmental sensors for rapid screening                                                                                                                                                                                                                                                                                                | Advanced continuous excitation chlorophyll fluorometer                                                                        | Rapid screening chlorophyll fluorometer                                                                                       | Hand-held fluorometer                                                                                                         |
| Main application                   | Fast and easy physiological diagnostics, stress prediction and monitoring, for field and laboratory use by professional and nonprofessional users                                                                                                                                                                                                                                         | Fast and easy physiological diagnostics, stress prediction and monitoring, for field and laboratory use by professional users | Fast and easy physiological diagnostics, stress prediction and monitoring, for field and laboratory use by professional users | Fast and easy physiological diagnostics, stress prediction and monitoring, for field and laboratory use by professional users |
| Measured and calculated parameters | Full OJIP kinetics, and 40 fluorescence parameters (Firmware can be customised to calculate over 60 parameters upon user request)                                                                                                                                                                                                                                                         | Full OJIP kinetics and over 50 fluorescence parameters                                                                        | Full OJIP kinetics and over 50 fluorescence parameters                                                                        | Partial OJIP, NPQ, and LC (light curve)                                                                                       |
| OJIP kinetics analysis             | Yes                                                                                                                                                                                                                                                                                                                                                                                       | Yes                                                                                                                           | Yes                                                                                                                           | Does not show full OJIP curve                                                                                                 |
| Environmental sensors              | PAR, temperature, humidity, atmospheric pressure                                                                                                                                                                                                                                                                                                                                          | No                                                                                                                            | No                                                                                                                            | No                                                                                                                            |
| Stress visualization               | Plant physiological status indicator on the screen with thresholds established by the user                                                                                                                                                                                                                                                                                                | No                                                                                                                            | No                                                                                                                            | No                                                                                                                            |
| Measurement time                   | 1 s (adjustable)                                                                                                                                                                                                                                                                                                                                                                          | 0.1–300 s                                                                                                                     | 1, 3 or 10 s                                                                                                                  | Adjustable                                                                                                                    |
| Excitation light                   | Blue LED, 430 nm                                                                                                                                                                                                                                                                                                                                                                          | Red LEDs, 650 nm                                                                                                              | Red LED, 627 nm                                                                                                               | Blue LED, 470 nm                                                                                                              |
| Maximum light intensity            | ~4,000 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$                                                                                                                                                                                                                                                                                                                       | Up to 3,500 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$                                                      | Up to 3,500 $\mu\text{mol}(\text{photon}) \text{ m}^{-2} \text{ s}^{-1}$                                                      | Up to 3,000 $\mu\text{E}$                                                                                                     |
| Detector                           | PIN photodiode with 690-nm filter                                                                                                                                                                                                                                                                                                                                                         | PIN photodiode with RG9                                                                                                       | PIN photodiode with RG9                                                                                                       | PIN photodiode with 697–750-nm filters                                                                                        |
| Data communication                 | Wi-Fi                                                                                                                                                                                                                                                                                                                                                                                     | USB 2.0                                                                                                                       | Bluetooth                                                                                                                     | Bluetooth, USB, or serial cable                                                                                               |
| Dimensions                         | 95 × 58 × 23 mm                                                                                                                                                                                                                                                                                                                                                                           | 170 × 85 × 40 mm                                                                                                              | 175 × 75 × 35 mm                                                                                                              | 120 × 57 × 30 mm                                                                                                              |
| Weight                             | 80 g                                                                                                                                                                                                                                                                                                                                                                                      | 565 g                                                                                                                         | 250 g                                                                                                                         | 180 g                                                                                                                         |
| Data storage                       | CSV export <i>via</i> Wi-Fi, unlimited recordings                                                                                                                                                                                                                                                                                                                                         | Up to 999 recordings, export <i>via</i> USB                                                                                   | Up to 200 recordings, export <i>via</i> Bluetooth                                                                             | Up to 100,000 data points, export <i>via</i> USB                                                                              |
| Dark adaptation                    | Optional, supported by "Kalaji Factor" correction                                                                                                                                                                                                                                                                                                                                         | Required for reliable $F_v/F_m$                                                                                               | Required for reliable $F_v/F_m$                                                                                               | Required for $Q_y/F_v/F_m$                                                                                                    |
| User interface                     | ECG-like visualisation and simplified interpretation                                                                                                                                                                                                                                                                                                                                      | Advanced research-oriented interface                                                                                          | Simple one-button operation                                                                                                   | Simple two-button operation                                                                                                   |
| Target users                       | Scientists, farmers, horticulture, forestry, non-specialists                                                                                                                                                                                                                                                                                                                              | Mainly researchers and advanced users                                                                                         | Researchers and screening programs                                                                                            | Basic laboratory and field users                                                                                              |
| Distinctive innovation             | Records up to 200,000 fluorescence data points during a 1-s induction measurement. Data can be downloaded and analysed without Bluetooth, connection cables, dedicated software, or a computer. The integrated online platform provides immediate data analysis and visualisation, including the fluorescence induction curve and a spider plot of the measured and calculated parameters | Advanced customizable protocols and high-resolution OJIP analysis. Modified protocols can be uploaded to the instrument       | Ultra-portable rapid screening                                                                                                | Low-cost simple fluorescence measurements                                                                                     |

including  $F_v/F_m$ , can be determined without the need for traditional, time-consuming dark adaptation.

Recently, the Kalaji Factor has been developed according to this protocol and is embedded in the firmware of the *PlantECG* fluorometer. It is based on a comparison between chlorophyll fluorescence measurements taken under ambient light and after dark adaptation of the same

samples and has been tested on different parts of dozens of plant species under different light conditions and at various times of day. The user takes at least 30 readings from representative samples and performs the measurements under both light and dark conditions. A calculation is then made to determine the percentage difference between the two values, which is entered into the fluorometer

as the Kalaji Factor before conducting subsequent measurements under ambient light. This is calculated according to the following equation:

$$\text{Kalaji Factor} = \text{ChF}_{\text{dark}} / \text{ChF}_{\text{light}}$$

Examples:  $0.83/0.75 = 1.11$ ;  $0.83/0.78 = 1.06$ ;  $0.83/0.73 = 1.14$ . This calculated value is entered on the device's website and then automatically used to multiply subsequent light-adapted readings; it typically ranges from 1.05 to 1.2, bringing measurements taken under ambient light closer to those expected from dark-adapted samples.

The application of this factor is more reliable when comparing plants (e.g., control vs. stress or treatment groups) measured under the same environmental conditions.

The comparability of results obtained with different fluorometers is a matter of fundamental importance for the interpretation of published data and for the conduct of multi-centre studies. It has been demonstrated that different devices may generate significantly different values for the same fluorescence parameters, owing to differences in actinic light sources, detector sensitivity, signal processing algorithms, and dark-adaptation systems (Murchie and Lawson 2013, Kalaji *et al.* 2017). The lack of standardisation in chlorophyll fluorescence measurements poses a serious challenge to the comparability of scientific results (Bussotti *et al.* 2011). Previous comparative studies of fluorometers have revealed both strong correlations of  $F_v/F_m$  values between devices from different manufacturers and significant differences in absolute parameter values, particularly  $PI_{\text{ABS}}$  (Brestic and Zivcak 2013, Kalaji *et al.* 2016). These findings underline the need for validation of new devices against reference instruments on a case-by-case basis, especially when they are intended for use in scientific research.

Among horticultural plants, susceptibility to drought stress is particularly pronounced in species with high water requirements, such as pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), and strawberry (*Fragaria × ananassa* Duch.). Seedlings of these species, due to their poorly developed root systems, are especially vulnerable to water deficit at early growth stages. It has been shown that even brief drought episodes at the seedling stage can permanently disrupt the photosynthetic performance of the plant and reduce yields (Cao *et al.* 2024).

The aim of this study was to validate the suitability of the new *PlantECG* fluorometer with three established measuring devices (*PocketPEA*, *HandyPEA*, *FluorPen FP 100*) for monitoring the photosynthetic performance of plants under progressive drought stress. The specific research objectives were: (1) to assess the dynamics of changes in the maximum quantum yield of PSII ( $F_v/F_m$ ) and the performance index ( $PI_{\text{ABS}}$ ) as measured by four fluorometers in pepper, tomato, and strawberry at successive stages of drought stress induced by the cessation of irrigation; (2) to analyse the agreement and correlation of results obtained with the *PlantECG* device relative to the reference fluorometers (*PocketPEA*, *HandyPEA*,

*FluorPen FP 100*); (3) to evaluate the suitability of the *PlantECG* as a tool for rapid diagnostic assessment of plant photosynthetic condition under stress conditions; and (4) to determine the species-specific photosynthetic response to water deficit in three horticultural species. We hypothesise that the *PlantECG* fluorometer generates chlorophyll fluorescence parameter values ( $F_v/F_m$  and  $PI_{\text{ABS}}$ ) and OJIP induction curves that are highly correlated with those obtained by fluorometers already well established on the market (*PocketPEA*, *HandyPEA*, and *FluorPen FP 100*), and that it is capable of detecting significant changes in the photosynthetic status of horticultural seedlings induced by progressive drought stress.

## Materials and methods

**Experimental design:** In this experiment, pepper (*Capsicum annuum* L.), cultivar "Palermo", strawberry (*Fragaria × ananassa* Duch.), cultivar "Grandarosa", and tomato (*Solanum lycopersicum* L.), cultivar "Rosamunda", were tested. These species were deliberately chosen as representing three different rates and strategies of response to water deficit among horticultural crops with high water requirements. Pepper is relatively tolerant, tomato is intermediate, and strawberry is most sensitive because of its shallow root system. This allowed the performance of fluorometers to be assessed across a broad range of stress dynamics. Plants were grown in a controlled greenhouse in pots filled with an organic substrate. One representative leaf was sampled from each of nine randomly chosen plants for each measurement. The average day/night temperatures were 26/18°C, with a photoperiod of 16 h light/8 h dark. The maximum photosynthetically active radiation (PAR) was about 500  $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ .

**Measurements:** Chlorophyll fluorescence measurements were performed for the first time on 9 September 2025 (0 h). From that point on, the plants were subjected to drought stress (no access to water). Subsequent measurements were taken 12, 24, 48, 72, and 96 h after the first measurement. All measurements were performed on the same nine chosen leaves at each time point using four fluorometers: *PlantECG*, *FluorPen FP 100* (*Photon Systems Instruments, spol. s r.o.*, Drásov, Czech Republic), *PocketPEA*, and *HandyPEA* (both *Hansatech*, UK). Two parameters were measured:  $F_v/F_m$  (by all four fluorometers) and  $PI_{\text{ABS}}$  (by all fluorometers, except *FluorPen FP 100*, which does not calculate this parameter). All measurements were performed at room temperature after 30 min of dark adaptation of the samples in the darkroom to ensure comparability with the reference devices. At the beginning of the experiment (0 h), one representative, fully developed leaf was selected from each plant and marked for identification. This same marked leaf was measured repeatedly at every subsequent sampling time (0, 12, 24, 48, 72, and 96 h). At each sampling time, the marked leaf was measured sequentially using all four fluorometers, with measurements taken at adjacent, previously unmeasured spots on the leaf blade

to minimise spatial variability while avoiding repeated excitation of the same tissue area.  $F_v/F_m$  and  $PI_{ABS}$  parameters were selected for comparison because they are the only parameters that can be derived, using directly comparable calculation principles, from tested devices; the remaining JIP-test parameters are not consistently available across instruments and were therefore excluded from this inter-device comparison.

**Statistical analysis:** A repeated-measures analysis of variance (*ANOVA*), with sampling time as the within-subject factor and fluorometer as the fixed factor, was used to test the effects of water deficiency on the values of these two measured parameters. *Fisher's* test was used to compare the means of the samples measured by the tested fluorometers at the 5% level of significance. The slopes and intercepts of the regression lines expressing the relationships between values measured by *PlantECG* and other fluorometers were analysed using the test of homogeneity of regression coefficients and compared at the 5% level of significance.

## Results

**Maximum quantum yield of PSII ( $F_v/F_m$ ) and performance index ( $PI_{ABS}$ ) in pepper plants:** Under optimal conditions (time point 0 h),  $F_v/F_m$  values measured in pepper plants by the *PlantECG*, *PocketPEA*, and *HandyPEA* fluorometers were at a similar level of 0.81–0.82 rel. u. (Fig. 2A). In contrast, *FluorPen FP 100*

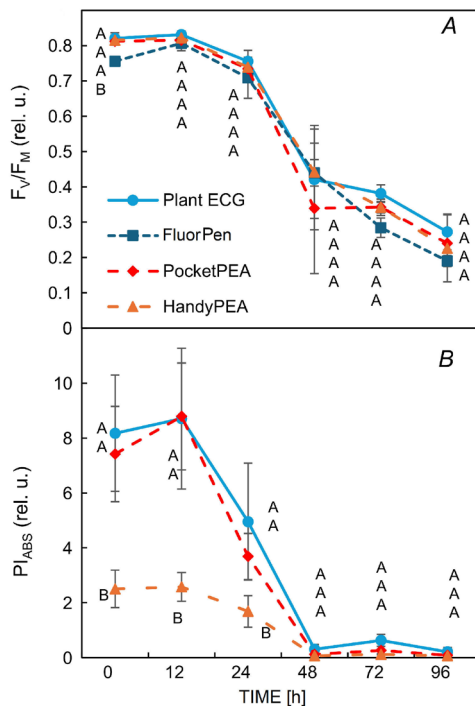


Fig. 2. Changes in maximum quantum yield of photosystem II ( $F_v/F_m$ ) (A) and performance index ( $PI_{ABS}$ ) (B) measured on pepper by different fluorometers. Mean values  $\pm$  S.D. Asterisk symbols indicate values that are significantly different in one term ( $n = 9$ ,  $p < 0.05$ ).

recorded significantly lower values (0.75 rel. u.). At 12 h,  $F_v/F_m$  values for all fluorometers ranged from 0.80 to 0.83 rel. u. and formed a single homogeneous group. A clear reduction in  $F_v/F_m$  was observed after 24 h of drought, when this index decreased to approximately 0.72–0.76 rel. u. across all devices, still without statistically significant differences between fluorometers. The greatest decline occurred between 24 and 48 h of stress. At 48 h,  $F_v/F_m$  values fell to the range of 0.33–0.42 rel. u., with no statistically significant differences between devices. In subsequent time points (72 h and 96 h),  $F_v/F_m$  values continued to decrease, reaching 0.19–0.28 rel. u. at 96 h. At these time points, all devices formed a single homogeneous group, which confirms that methodological differences between fluorometers become negligible under deeper stress.

$PI_{ABS}$  values measured in pepper showed considerably greater variation between fluorometers than  $F_v/F_m$ , confirming the higher sensitivity of this parameter to methodological differences between devices (Fig. 2B). At baseline (0 h), *PlantECG* and *PocketPEA* recorded  $PI_{ABS}$  values at a similar level (8.1 and 7.7 rel. u., respectively), while *HandyPEA* showed significantly lower values (2.3 rel. u.). At 12 h, *PlantECG* (8.1 rel. u.) and *PocketPEA* (8.5 rel. u.) maintained values close to baseline or slightly increased, whereas *HandyPEA* remained consistently low (2.3 rel. u.). A sharp decline in  $PI_{ABS}$  occurred after 24 h: *PlantECG* recorded a value of 4.8 rel. u., and *PocketPEA* recorded 3.8 rel. u., while *HandyPEA* dropped to approximately 1.8 rel. u.  $PI_{ABS}$  values for all devices reached levels close to zero at 48 h and remained low until the end of the experiment (72 h and 96 h), no longer significantly differentiating the individual fluorometers.

**Maximum quantum yield of PSII ( $F_v/F_m$ ) and performance index ( $PI_{ABS}$ ) in strawberry plants:** Baseline  $F_v/F_m$  values measured by all fluorometers were similar, ranging from 0.79 to 0.83 rel. u. at 0 h and 12 h, without significant differentiation between devices (Fig. 3A). A significant reduction in  $F_v/F_m$  occurred between 12 and 24 h without watering, considerably earlier than in pepper and tomato. At 24 h,  $F_v/F_m$  values across all devices decreased to 0.15–0.22 rel. u., with no statistically significant differences between fluorometers. At 48 h, statistically significant differences between instruments emerged. *PlantECG*, *PocketPEA*, and *HandyPEA* recorded markedly higher values (0.12–0.17 rel. u.), while *FluorPen FP 100* reached values close to zero (0.01 rel. u.). At 72 h, *PlantECG* showed significantly higher values (0.19 rel. u.) than *FluorPen FP 100*, *PocketPEA*, and *HandyPEA* (0.05–0.11 rel. u.). At the final time point (96 h),  $F_v/F_m$  values for all devices converged to 0.01–0.05 rel. u., with no differences between them.

As in pepper,  $PI_{ABS}$  values measured in strawberry plants by *PlantECG* were significantly higher than those obtained by the other fluorometers throughout the entire period in which this parameter was measurable (Fig. 3B).  $PI_{ABS}$ , measured at 0 h by *PlantECG*, was 20.0 rel. u., *PocketPEA* at 11.5 rel. u., and *HandyPEA* at 3.5 rel. u. Such large differences in  $PI_{ABS}$  values between devices

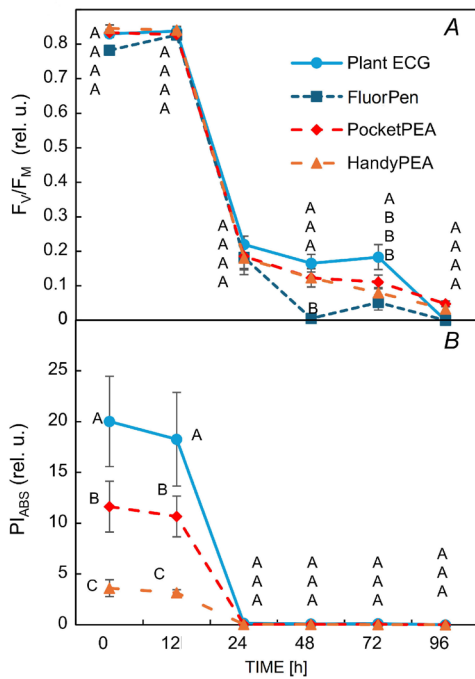


Fig. 3. Changes in maximum quantum yield of photosystem II ( $F_v/F_m$ ) (A) and performance index ( $PI_{ABS}$ ) (B) measured on strawberry by different fluorimeters. Mean values  $\pm$  S.D. Asterisk symbols indicate values that are significantly different in one term ( $n = 9$ ,  $p < 0.05$ ).

were statistically significant and represent one of the key findings of this study, confirming the hypothesis that absolute  $PI_{ABS}$  values are not directly comparable between fluorimeters. At 12 h,  $PI_{ABS}$  values for *PlantECG* (18.5 rel. u.) and *PocketPEA* (10.5 rel. u.) remained at baseline levels, while *HandyPEA* values remained consistently low (3.0 rel. u.). At 24 h, values for all devices dropped to near zero, forming a single homogeneous group, and this state persisted throughout all subsequent measurement time points (48–96 h).

**Maximum quantum yield of PSII ( $F_v/F_m$ ) and performance index ( $PI_{ABS}$ ) in tomato plants:** Tomato seedlings exhibited intermediate drought stress tolerance, between the relatively tolerant pepper and the sensitive strawberry (Fig. 4A). Baseline  $F_v/F_m$  values were similar across all fluorimeters and ranged from 0.75 to 0.81 rel. u. at time points 0 h and 12 h, forming a single homogeneous group at all time points. A slight reduction in this parameter was observed at 24 h (0.77–0.81 rel. u.), although differences between devices remained nonsignificant. All fluorimeters belonged to the same homogeneous group. At 48 h, values declined to 0.65–0.79 rel. u., with *PlantECG* recording the highest values (0.79) and *PocketPEA* the lowest (0.65), yet these differences were still not statistically significant. At 72 h, values reached the level of 0.33–0.55 rel. u. and, as at earlier time points, no statistically significant differences between individual instruments were found. At the final time point (96 h),  $F_v/F_m$  values for all fluorimeters ranged from 0.08 to 0.23 rel. u., with *PlantECG* showing the highest values

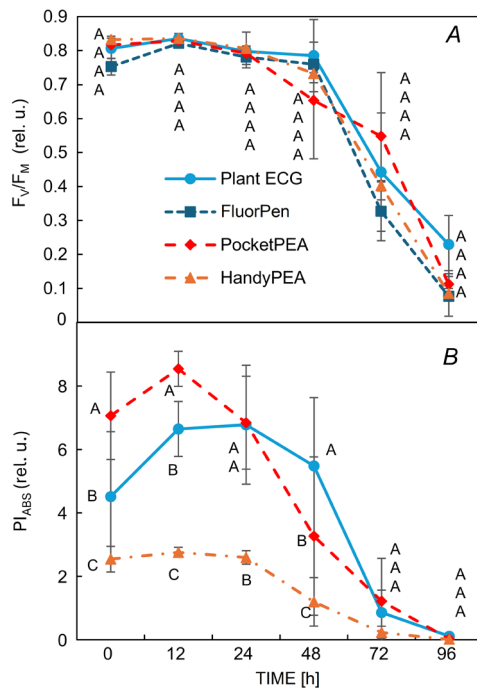


Fig. 4. Changes in maximum quantum yield of photosystem II ( $F_v/F_m$ ) (A) and performance index ( $PI_{ABS}$ ) (B) measured on tomato by different fluorimeters. Mean values  $\pm$  S.D. Asterisk symbols indicate values that are significantly different in one term ( $n = 9$ ,  $p < 0.05$ ).

(0.23) and the remaining devices below 0.15 rel. u. However, even at this time point, no significant differences between instruments were detected.

The dynamics of  $PI_{ABS}$  changes in tomato were considerably more differentiated between individual fluorimeters than was the case for  $F_v/F_m$  (Fig. 4B). At 0 h, *PocketPEA* recorded the highest values (7.0 rel. u.), *PlantECG* intermediate values (4.5 rel. u.), and *HandyPEA* the lowest (2.3 rel. u.). At 12 h,  $PI_{ABS}$  values measured by *PocketPEA* reached their maximum (8.5 rel. u.), *PlantECG* showed approximately 6.5 rel. u., and *HandyPEA* 2.5 rel. u. At 24 h, the differentiation between devices remained: *PocketPEA* and *PlantECG* showed similar values (6.5–7.0 rel. u.), while *HandyPEA* recorded a significantly lower value (2.3 rel. u.). At 48 h,  $PI_{ABS}$  values measured by *PlantECG* were 5.5 rel. u. and by *PocketPEA* 3.0 rel. u., with no statistically significant differences between these two instruments. In contrast, the value measured by *HandyPEA* was 1.3 rel. u. and was significantly lower than values recorded by the other two instruments. It is worth noting that at time points 0–48 h, *PocketPEA* consistently recorded higher values than *PlantECG*, a trend opposite to that observed in pepper and strawberry. At 72 h and 96 h, a sharp decline in  $PI_{ABS}$  was observed across all devices (to 0.1–1.5 rel. u.), and differences between fluorimeters ceased to be statistically significant. All instruments formed a single homogeneous group. The maintenance of measurable  $PI_{ABS}$  values for a longer period, combined with the slower rate of  $F_v/F_m$  decline, confirms the greater adaptive capacity of tomato seedlings to progressive water deficit.

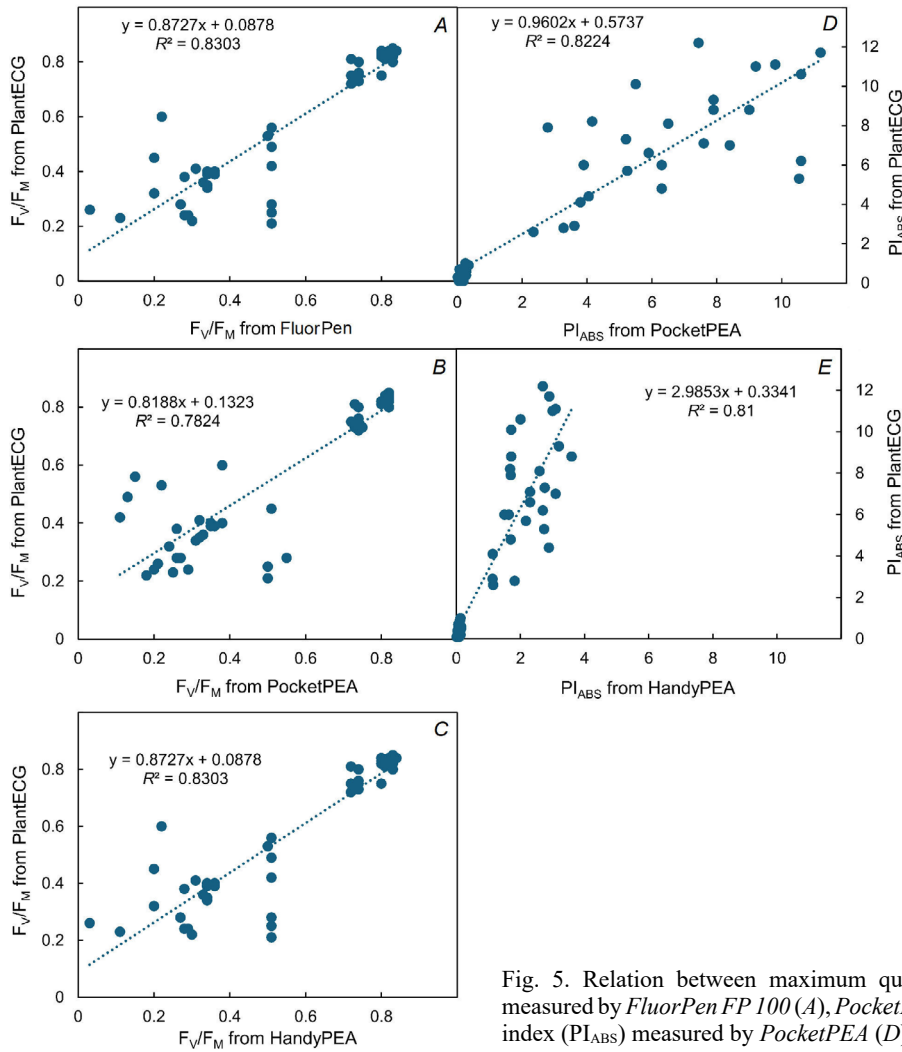


Fig. 5. Relation between maximum quantum yield of photosystem II ( $F_v/F_m$ ) measured by *FluorPen FP 100* (A), *PocketPEA* (B), *HandyPEA* (C), and performance index ( $PI_{ABS}$ ) measured by *PocketPEA* (D) and *HandyPEA* (E) in pepper.

#### Correlations between *PlantECG* and other fluorometers

**in pepper:** Fig. 5 presents the results of linear regression analysis of  $F_v/F_m$  and  $PI_{ABS}$  values measured by *PlantECG* as a function of values obtained by reference fluorometers in pepper seedlings. The results indicate a moderate to strong correlation for  $F_v/F_m$ , and an even stronger correlation for  $PI_{ABS}$ , which is somewhat surprising given the higher variability of absolute  $PI_{ABS}$  values between devices. The linear relationship described by the equation  $y = 0.8727x + 0.0878$  with  $R^2 = 0.8303$  indicates good agreement between the two devices in measuring  $F_v/F_m$  (Fig. 5A). A regression slope below 1 (0.873) indicates that *PlantECG* systematically overestimated  $F_v/F_m$  values relative to *FluorPen FP 100*, particularly at low values (advanced stress). Noticeable scatter of points was observed in the 0.20–0.50 value range, corresponding to the 48 h and 72 h time points.

The correlation between  $F_v/F_m$  values measured by *PlantECG* and *PocketPEA* (Fig. 5B) is slightly weaker ( $R^2 = 0.7824$ ,  $y = 0.8188x + 0.1323$ ), with a visible tendency for *PlantECG* to overestimate  $F_v/F_m$  at low *PocketPEA* readings. The higher intercept (0.132) reflects

a systematic offset between devices, most apparent in the low  $F_v/F_m$  range, *i.e.*, under deep stress.

The regression equation for *PlantECG* and *HandyPEA* results (Fig. 5C) is identical to that for *FluorPen FP 100* ( $y = 0.8727x + 0.0878$ ,  $R^2 = 0.8303$ ), indicating that *HandyPEA* and *FluorPen FP 100* generated virtually identical  $F_v/F_m$  values in pepper, with a comparable relationship to *PlantECG*.

The correlation for  $PI_{ABS}$  measured by *PlantECG* and *PocketPEA* was strong ( $R^2 = 0.8224$ ,  $y = 0.9602x + 0.5737$ ) (Fig. 5D). A slope close to 1 and a small intercept suggest that *PlantECG* and *PocketPEA* recorded  $PI_{ABS}$  values on a similar absolute scale in pepper, although point scatter in the mid-range was considerable.

The correlation of  $PI_{ABS}$  measured by *PlantECG* and *HandyPEA* (Fig. 5E) was equally strong ( $R^2 = 0.81$ ), but the regression slope of 2.985 indicates that *HandyPEA* recorded  $PI_{ABS}$  values nearly three times lower than *PlantECG* and *PocketPEA* at the same plant conditions (Fig. 5D,E). This reflects fundamental incomparability of absolute  $PI_{ABS}$  values between these devices, while maintaining a strong correlation in the direction of

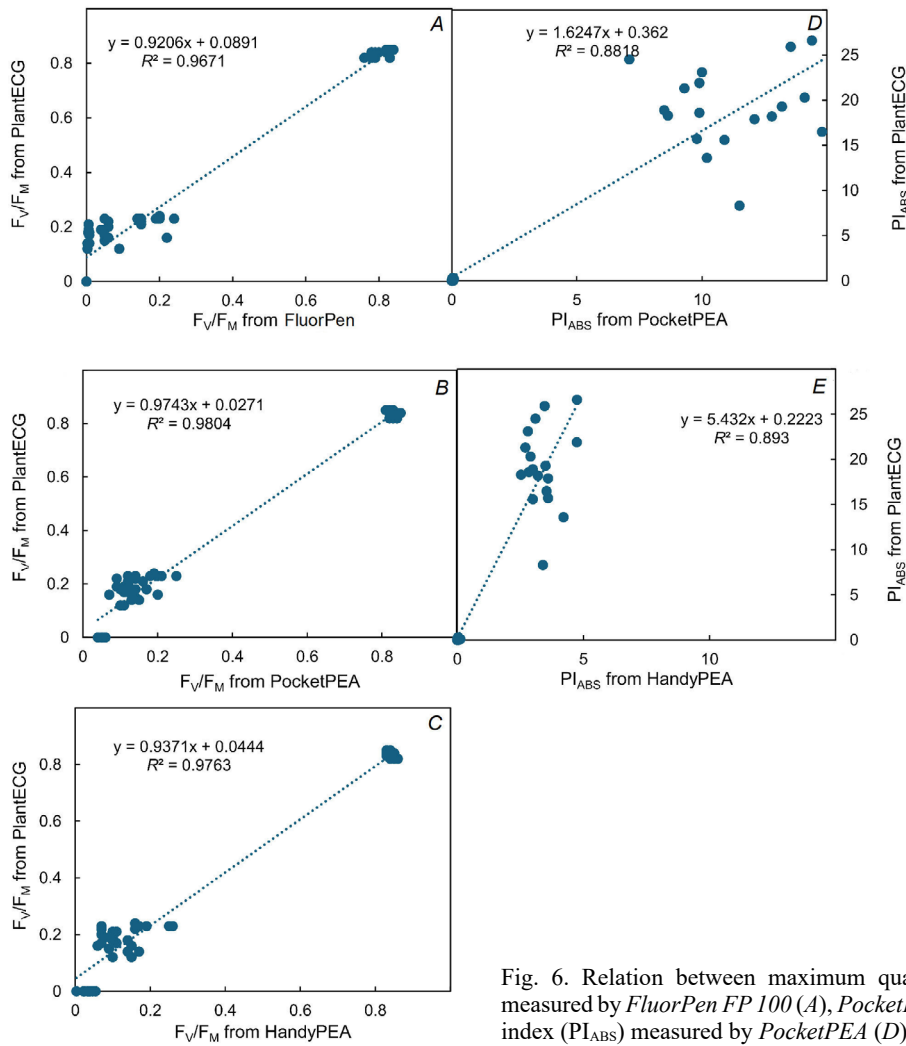


Fig. 6. Relation between maximum quantum yield of photosystem II ( $F_v/F_m$ ) measured by *FluorPen FP 100* (A), *PocketPEA* (B), *HandyPEA* (C) and performance index ( $PI_{ABS}$ ) measured by *PocketPEA* (D) and *HandyPEA* (E) in strawberry.

change – a key finding for the validation of *PlantECG* as a diagnostically useful fluorometer.

**Correlations between *PlantECG* and reference fluorimeters in strawberry:** Fig. 6 illustrates the correlation relationships in strawberry – the species that showed the most rapid response to drought stress. A characteristic feature of the correlation plots for this species is the clustering of data points into two clearly separated clusters: one near high values (time points 0 h and 12 h,  $F_v/F_m$  fluctuated from 0.80 to 0.84) and another near zero (time points 24–96 h), reflecting the abrupt collapse of photosynthetic activity after 24 h of drought.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *FluorPen FP 100* (Fig. 6A) revealed a very strong correlation ( $R^2 = 0.9671$ ,  $y = 0.9206x + 0.0891$ ), with a slope close to 1 and a minimal intercept.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *PocketPEA* (Fig. 6B) revealed an equally strong correlation ( $R^2 = 0.9804$ ,  $y = 0.9743x + 0.0271$ ) – the highest  $R^2$  among all  $F_v/F_m$  correlation plots. A slope close to 1 and an intercept near zero suggest near-perfect agreement between *PlantECG* and *PocketPEA* in measuring  $F_v/F_m$  in

strawberry. Such a high  $R^2$  confirms *PlantECG*'s ability to detect significant, physiologically meaningful changes in this parameter.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *HandyPEA* (Fig. 6C) revealed a strong correlation ( $R^2 = 0.9763$ ,  $y = 0.9371x + 0.0444$ ), like the relationship with *PocketPEA*, with a slightly lower slope and intercept. These results confirm that *PlantECG* shows very good agreement with *Hansatech* instruments in detecting  $F_v/F_m$  changes in species exhibiting a rapid stress response.

Analysis of  $PI_{ABS}$  values measured by *PlantECG* and *PocketPEA* (Fig. 6D) revealed a strong correlation ( $R^2 = 0.8818$ ,  $y = 1.6247x + 0.362$ ), but the regression slope of 1.625 indicates that *PlantECG* systematically recorded  $PI_{ABS}$  values approximately 60% higher than *PocketPEA*. This is consistent with the pepper results and confirms the systematic overestimation of  $PI_{ABS}$  by *PlantECG* or a different scale for this parameter in the device.

Analysis of  $PI_{ABS}$  values measured by *PlantECG* and *HandyPEA* (Fig. 6E) revealed the highest regression slope among all  $PI_{ABS}$  correlations, with  $R^2 = 0.893$  and  $y = 5.432x + 0.2223$ . This means that *PlantECG* generates  $PI_{ABS}$  values more than five times higher than *HandyPEA*,

which, combined with a strong correlation, confirms that although both devices measure the same physiological phenomenon, they employ fundamentally different calculation algorithms or calibrations. This finding serves as an important warning for researchers comparing data from different fluorimeters without prior cross-validation.

**Correlations between *PlantECG* and reference fluorimeters in tomato:** Fig. 7 presents the correlations for tomato, a species of intermediate drought-stress sensitivity and the most gradual rate of change in both parameters. Due to the slower rate of response, data points on the correlation plots are more evenly distributed along the axes, providing a more reliable assessment of correlation quality than in strawberry.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *FluorPen FP 100* (Fig. 7A) revealed a strong correlation ( $R^2 = 0.8823$ ,  $y = 0.7984x + 0.1799$ ), with a slope clearly below 1 (0.798) and a relatively high intercept (0.18). This indicates that *PlantECG* showed systematically higher  $F_v/F_m$  values than *FluorPen FP 100*, particularly at low readings from the latter (advanced stress time points). Several outlier data points are visible, which may

reflect natural variation in the physiological conditions of individual plants.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *PocketPEA* (Fig. 7B) revealed the weakest correlation for this parameter among all comparisons in tomato ( $R^2 = 0.7207$ ,  $y = 0.7653x + 0.1697$ ). The pronounced scatter of data points suggests that in tomato, *PlantECG* and *PocketPEA* show greater discrepancy than other device pairs, which may be related to differences in detector sensitivity between the two instruments in the intermediate  $F_v/F_m$  range (0.40–0.75 rel. u.) characteristic of moderate stress.

Analysis of  $F_v/F_m$  values measured by *PlantECG* and *HandyPEA* (Fig. 7C) revealed a strong correlation ( $R^2 = 0.8836$ ,  $y = 0.7884x + 0.1628$ ), nearly identical to the *PlantECG*–*FluorPen FP 100* relationship. This again confirmed the mutual convergence of *HandyPEA* and *FluorPen FP 100* results, alongside a systematic overestimation of  $F_v/F_m$  by *PlantECG* relative to both devices.

Analysis of  $PI_{ABS}$  values measured by *PlantECG* and *HandyPEA* (Fig. 7D) revealed a moderate correlation ( $R^2 = 0.671$ ,  $y = 2.0931x + 0.7428$ ) – the weakest for  $PI_{ABS}$

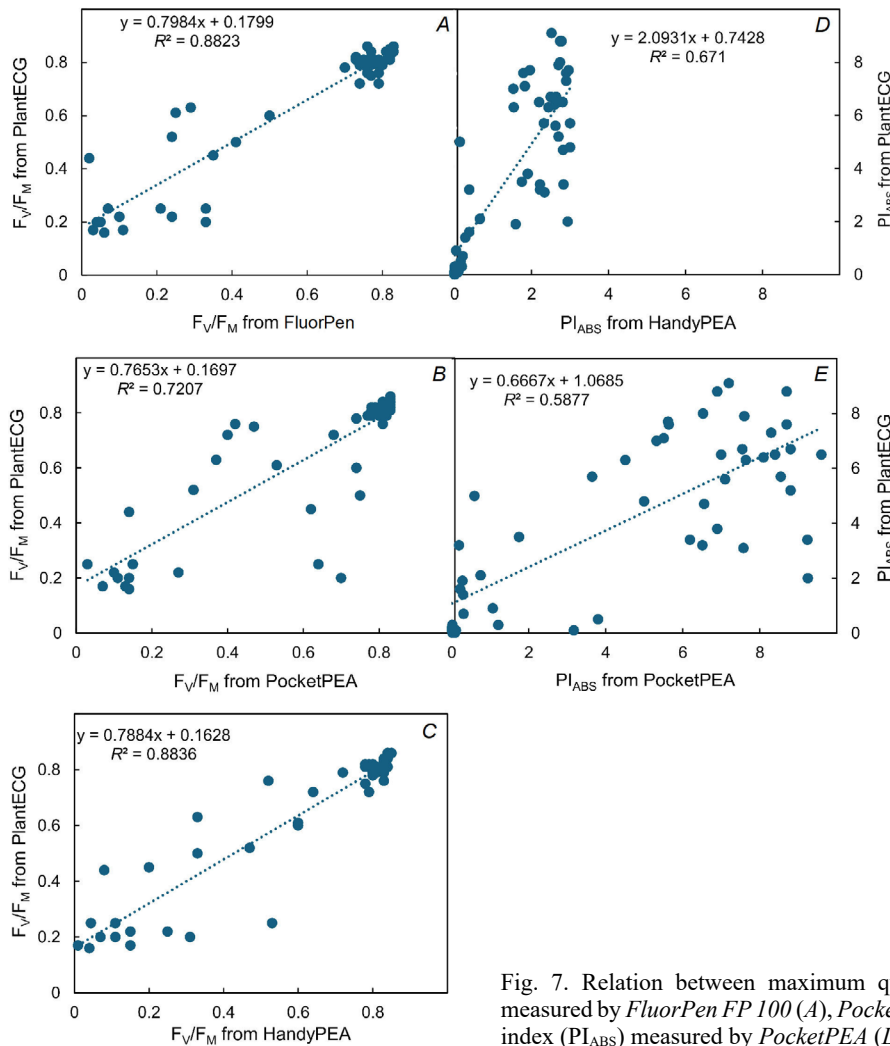


Fig. 7. Relation between maximum quantum yield of photosystem II ( $F_v/F_m$ ) measured by *FluorPen FP 100* (A), *PocketPEA* (B), *HandyPEA* (C) and performance index ( $PI_{ABS}$ ) measured by *PocketPEA* (D) and *HandyPEA* (E) in tomato.

in the entire dataset. The large scatter of data points and low  $R^2$  indicate that in tomato, under conditions of gradual stress and intermediate  $PI_{ABS}$  values, the agreement between *PlantECG* and *HandyPEA* for this parameter is limited.

Analysis of  $PI_{ABS}$  values measured by *PlantECG* and *PocketPEA* (Fig. 7E) revealed the lowest  $R^2$  among all analysed correlation plots ( $R^2 = 0.5877$ ,  $y = 0.6667x + 1.0685$ ). A slope below 1 and a high intercept indicate a lack of linear proportionality between  $PI_{ABS}$  values recorded by both devices under conditions of gradual, moderate drought stress in tomato. Such a low coefficient of determination is a surprising result considering the strong correlations obtained for the same parameter in strawberry ( $R^2 = 0.88$ ), suggesting that *PlantECG*'s ability to reflect  $PI_{ABS}$  changes consistent with *PocketPEA* is species-specific and depends on the rate and nature of the physiological stress response.

## Discussion

The research hypothesis assumed that *PlantECG* would generate results highly correlated with those of the reference devices (*PocketPEA*, *HandyPEA*, and *FluorPen FP 100*), with possible differences in absolute values, particularly for  $PI_{ABS}$ . The results of the present study largely confirmed this hypothesis, with important species-specific caveats.

The obtained results confirm that the maximum quantum yield of PSII ( $F_v/F_m$ ) was a relatively stable parameter across all four fluorometers, which is consistent with findings from earlier comparative studies (Röttgers 2007, Padhi *et al.* 2021). In all three analysed species – pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), and strawberry (*Fragaria × ananassa* Duch.), baseline  $F_v/F_m$  values measured by *PlantECG*, *PocketPEA*, *HandyPEA*, and *FluorPen FP 100* were at a comparable, physiologically optimal level (approximately 0.80–0.83 rel. units), close to reference values reported in the literature for plants in good physiological conditions (Björkman and Demmig 1987, Strasser *et al.* 2004). An exception was *FluorPen FP 100*, which recorded markedly lower values in pepper already at the baseline measurement (0.75 rel. units). This discrepancy may reflect methodological differences between devices, such as differences in optical geometry or excitation wavelength, rather than a single, definitive cause; similar device-dependent variation in baseline fluorescence readings has been reported when comparing *PEA*-type instruments using excitation light of different wavelengths (Padhi *et al.* 2021).

The dynamics of  $F_v/F_m$  changes under progressive drought stress were similar across all fluorometers, although with varying intensity depending on species. Strawberry seedlings showed the fastest and most pronounced response: a sharp decline in  $F_v/F_m$  already between 12 and 24 h without irrigation points to the sensitivity of this species to water deficit, which corresponds to findings from other studies emphasising the limited drought tolerance of *Fragaria × ananassa* (commercial strawberries) (Blanke and Cooke 2004, Nezhadahmadi *et al.* 2013). Pepper

showed the greatest tolerance. A marked decline in  $F_v/F_m$  occurred only between 24 and 48 h, while absolute values during the first 12 h remained at levels suggesting no permanent or serious damage to the photosynthetic apparatus. Tomato exhibited an intermediate position, with a noticeable but less abrupt decrease in  $F_v/F_m$  over the first 48 h, consistent with the moderate drought tolerance of tomato described in the literature (Sánchez-Rodríguez *et al.* 2010).

Statistically significant differences between fluorometers in  $F_v/F_m$  were recorded only at selected time points, generally at intermediate values (0.3–0.6 rel. units). At low values (indicating deep stress), the measured values converged, suggesting the methodological sensitivity of the devices is most apparent in the intermediate range. This phenomenon was described by Lichtenthaler *et al.* (2005), who pointed out that under deep photoinhibition, the algorithms for determining  $F_0$  and  $F_m$  lose diagnostic significance, and differences between devices arising from the intensity and geometry of the saturating pulse become irrelevant. The significant differences observed at the 48-h time point between *PocketPEA*, *PlantECG*, and *FluorPen FP 100* in pepper may reflect differences in the way  $F_0$  is calculated, or differences in the duration and intensity of the saturation pulse. Divergence in  $F_0$  values between *PEA*-type devices using excitation light of different wavelengths (red vs. blue) has been documented by Padhi *et al.* (2021), who, comparing *HandyPEA* with *FluorPen FP 100*, demonstrated that absolute  $F_0$  values increase with excitation light intensity at a device-dependent rate, directly affecting calculated  $F_v/F_m$  values under moderate stress.

Regarding  $F_v/F_m$ , *PlantECG* showed satisfactory correlation with the reference devices across all three species ( $R^2 = 0.72$ – $0.98$ ), with the best agreement obtained for strawberry ( $R^2 > 0.97$  for both *FluorPen FP 100* and *PocketPEA*) and the weakest for tomato, particularly in relation to *PocketPEA* ( $R^2 = 0.72$ ). The weaker correlations in tomato suggest limitations when changes are slow and progressive, as biological variability overlaps with methodological differences.

The performance index ( $PI_{ABS}$ ) proved to be far more sensitive to methodological differences between fluorometers.  $PI_{ABS}$  values measured by *HandyPEA* were systematically lower than those recorded by *PlantECG* and *PocketPEA*. These discrepancies are in line with observations by Strasser *et al.* (2004), who emphasised that  $PI_{ABS}$ , as a composite index calculated from several fluorescence parameters simultaneously, is particularly susceptible to the propagation of methodological errors. This index integrates the efficiency of energy absorption, electron transport, and final acceptor reduction, so any difference in calibration, optical geometry, or signal processing algorithm between devices accumulates in the final  $PI_{ABS}$  value.

Linear regression analysis showed that despite the absolute differences in  $PI_{ABS}$  values, the relationships between *PlantECG* results and the reference fluorometers are strong and linear in pepper and strawberry ( $R^2 = 0.81$ – $0.89$ ). The exception was tomato, where  $PI_{ABS}$  correlations

were markedly weaker ( $R^2 = 0.59\text{--}0.67$ ), which may be attributable to the slower and more gradual nature of the physiological stress response in this species, generating a larger number of data points at intermediate values. As demonstrated by Yusuf *et al.* (2010), under gradual stress conditions,  $PI_{ABS}$  values exhibit higher intraspecific variability, which complicates linear modelling of relationships between devices. Consequently, the results do not support comparability of absolute  $PI_{ABS}$  values measured by tested devices:  $PI_{ABS}$  values cannot be directly compared with those from other fluorometers without prior calibration or the application of conversion equations.

The species-specific nature of the photosynthetic response to drought stress revealed in the present study is relevant from the perspective of practical applications of pulse fluorometry. Strawberry, as a species with a shallow root system and a high water demand (Blanke and Cooke 2004), showed the earliest decline in both  $F_V/F_M$  and  $PI_{ABS}$ . The maintenance of high and stable  $PI_{ABS}$  values in tomato during the first 48 h of stress, alongside a moderate decline in  $F_V/F_M$ , suggests that the mechanisms regulating electron transport in this species are more resistant to water deficit than processes on the antenna side. The dissociation between  $F_V/F_M$  and  $PI_{ABS}$  as indicators of PSII condition has been described by Tsimilli-Michael and Strasser (2008), who indicated that  $PI_{ABS}$  can detect sublethal damage to the photosynthetic apparatus earlier than  $F_V/F_M$ , which is relevant for precise monitoring of plant responses to abiotic stresses.

From a practical standpoint, the results of the present study indicate that *PlantECG* can be used as a tool for rapid diagnostic assessment and monitoring of photosynthetic condition trends in horticultural plants under drought stress. As a device designed not only for laboratory but also for field applications and rapid multi-point measurements, *PlantECG* fulfils the key criterion of diagnostic utility. It showed a high correlation of  $F_V/F_M$  trends with the reference devices. The validation approach based on assessing trend correlations rather than direct comparison of absolute values was applied by Bussotti *et al.* (2011) in field comparative studies of PEA fluorometers, demonstrating that  $F_V/F_M$  is stable and well correlated across devices and operators, whereas  $PI_{ABS}$  exhibits greater methodological variability and requires careful interpretation in inter-instrument comparisons. The  $PI_{ABS}$  parameter should therefore be interpreted with caution and exclusively in relation to the internal value scale of the device, without direct comparison with data from other fluorometers. Practical application of fluorometers in crop monitoring thus requires the development of species-specific correction equations, particularly for  $PI_{ABS}$ , which should be the objective of future validation studies covering a broader range of species and growing conditions. A dedicated analysis of relative, baseline-normalised changes in  $F_V/F_M$  and  $PI_{ABS}$ , aimed specifically at comparing the onset of stress detection across devices, was beyond the scope of the present validation study, but represents a valuable direction for future research specifically designed to benchmark *PlantECG*'s rapid stress-screening capability. Moreover, a broader evaluation

of *PlantECG*'s full JIP-test output is recommended as a direction for future studies.

**Conclusion:** The results of this study demonstrate that *PlantECG* reliably detected the progressive deterioration of photosynthetic performance induced by drought stress in pepper, tomato, and strawberry plants. The  $F_V/F_M$  values obtained with *PlantECG* were strongly correlated with those measured using *PocketPEA*, *HandyPEA*, and *FluorPen FP 100* across all three species, supporting the suitability of *PlantECG* for monitoring changes in the maximum quantum efficiency of PSII photochemistry. Nevertheless, some systematic differences were observed, particularly under advanced stress conditions, when *PlantECG* tended to record slightly higher ( $F_V/F_M$ ) values than some of the reference instruments.

The research hypothesis was only partially supported for  $PI_{ABS}$ . *PlantECG* showed strong correlations with *PocketPEA* and *HandyPEA* in pepper and strawberry, indicating a comparable ability to follow the direction and magnitude of drought-induced physiological changes. However, correlations were weaker in tomato, and the absolute  $PI_{ABS}$  values differed substantially among fluorometers. Therefore,  $PI_{ABS}$  values obtained with different instruments should not be considered directly interchangeable or compared without prior cross-validation.

Overall, the findings support the use of *PlantECG* as a reliable tool for rapid assessment of drought-induced changes in plant photosynthetic condition, particularly when measurements are interpreted relative to the internal scale and baseline values of the device. Further validation involving a broader range of species, stress intensities, environmental conditions, and independent datasets is required before robust device- and species-specific conversion equations can be established. Future studies should also evaluate the comparability of OJIP chlorophyll fluorescence induction curves, additional JIP-test parameters and determine the sensitivity of the tested fluorometers to the early onset of physiological stress.

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