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**REVIEW**

# Recent Advances and Emerging Trends in Chlorophyll Fluorescence Parameter $F_v/F_m$

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**ABSTRACT:** Chlorophyll fluorescence, particularly the parameter $F_v/F_m$, has emerged as a reliable, non-invasive indicator of the maximum quantum efficiency of Photosystem II (PSII) in plants. Over the past decade, significant research has leveraged $F_v/F_m$ to evaluate plant responses to a wide range of biotic and abiotic stresses, as well as to support crop improvement and ecological monitoring. This review synthesizes recent progress in understanding the physiological basis, measurement techniques, and applied significance of $F_v/F_m$ across diverse plant systems. We highlight methodological advancements in fluorescence imaging and remote sensing, identify consistent patterns and contrasting findings in stress-response studies, and explore how $F_v/F_m$ correlates with other physiological and molecular indicators. Despite its widespread use, challenges remain regarding data interpretation, environmental variability, and integration with multi-parameter models. This review also addresses critical knowledge gaps and proposes future research directions, including AI-driven analysis, a least-squares support vector machine (LSSVM) model, and high-resolution phenotyping. By consolidating findings from recent literature, this work provides a comprehensive reference for researchers aiming to harness $F_v/F_m$ in both basic and applied plant sciences.

**KEYWORDS:** Chlorophyll fluorescence; $F_v/F_m$; photosystem II stress

## 1 Introduction

Chlorophyll fluorescence has become an indispensable tool in plant physiology for evaluating the status and performance of the photosynthetic apparatus, particularly Photosystem II (PSII). When a plant is exposed to light, part of the absorbed energy is utilized in photochemistry, part is dissipated as heat, and the remainder is re-emitted as fluorescence. This re-emitted fluorescence, primarily arising from chlorophyll a in PSII, provides critical insight into the functioning and integrity of the photosynthetic machinery under both optimal and stress conditions [1]. Among the various fluorescence parameters, $F_v/F_m$—the maximum quantum efficiency of PSII—is one of the most widely utilized indicators. It is calculated from the difference between the maximum fluorescence ($F_m$) and the minimal fluorescence ($F_o$) of a dark-adapted leaf, expressed as $F_v/F_m = (F_m - F_o)/F_m$. Under optimal physiological conditions, $F_v/F_m$ typically ranges from 0.74 to 0.85, and deviations from this range are indicative of stress-induced photoinhibition or damage to PSII reaction centers [2].

The significance of $F_v/F_m$ lies in its simplicity and non-invasiveness, allowing researchers to assess the physiological status of plants quickly and accurately under a range of environmental conditions. Over the past decade, advancements in instrumentation such as Pulse-Amplitude Modulated (PAM) fluorometers and chlorophyll fluorescence imaging systems have enabled researchers to evaluate $F_v/F_m$ at high spatial and temporal resolutions, both in controlled environments and in the field. These technological innovations have facilitated its application in diverse domains, including crop breeding, ecosystem monitoring, and stress physiology [3]. From a mechanistic standpoint, $F_v/F_m$ is tightly linked to the efficiency of photochemical processes in PSII. A decline in this parameter reflects a disturbance in the energy transfer chain or damage to the PSII reaction center, often caused by abiotic stressors such as high light intensity, drought, salinity, heat, and cold. For example, a study by Faseela [4] emphasized that $F_v/F_m$ is among the earliest detectable indicators of stress before morphological symptoms become visible and thus offers a valuable early-warning system for plant health monitoring.

The application of $F_v/F_m$ in recent research has not been limited to stress detection alone. It has been used to evaluate genetic variability in stress tolerance across cultivars, making it a crucial parameter in plant breeding programs. Moreover, it is increasingly integrated with remote sensing technologies, particularly Unmanned Aerial Vehicles (UAVs) equipped with fluorescence sensors, to monitor crop performance across large spatial scales [5]. Despite its widespread utility, the interpretation of $F_v/F_m$ data is not without challenges. Variability in measurement protocols, diurnal fluctuations, and environmental interactions can all influence its accuracy and reproducibility. Additionally, while a decline in $F_v/F_m$ is generally indicative of stress, the precise cause and nature of the stress often require complementary measurements, such as Non-Photochemical Quenching (NPQ), gas exchange parameters, or molecular markers [1]. The foundational theory behind chlorophyll fluorescence has been well-established for decades [6]. Early work by Krause & Weis [7] and Bolhar-Nordenkampf et al. [8] laid the groundwork for understanding fluorescence dynamics, distinguishing between photochemical and non-photochemical pathways, and interpreting various fluorescence transients. Recent research has expanded upon this by integrating $F_v/F_m$ with systems biology approaches, including transcriptomics and metabolomics, to provide a more comprehensive view of stress responses.

By reviewing the progress made over the last decade, this paper aims to provide a comprehensive and critical overview of the role of $F_v/F_m$ in plant science. We highlight both the strengths and limitations of current approaches, underscore the parameter's versatility, and suggest ways in which future research can enhance its utility. Ultimately, this review seeks to serve as a reference point for researchers aiming to employ chlorophyll fluorescence and specifically $F_v/F_m$ as a diagnostic, predictive, and integrative tool in plant biology.

## 2 Fundamental Concepts and Theoretical Background

### *2.1 Basic Principles of Chlorophyll Fluorescence and PSII Photochemistry*

Chlorophyll fluorescence arises when chlorophyll molecules in plants absorb light energy, and a portion of this energy is re-emitted as fluorescence, rather than being used in photochemical reactions or dissipated as heat. This process is particularly informative when observing PSII, which is the core component of the photosynthetic apparatus responsible for the initial steps of light energy conversion.

PSII photochemistry involves the absorption of photons by the light-harvesting complexes, followed by energy transfer to the reaction center, where water is split to generate electrons, protons, and oxygen. The efficiency of this process can be modulated under environmental stresses, which in turn influences the intensity and dynamics of chlorophyll fluorescence [9]. Under stress conditions, impaired electron transport or photodamage to PSII leads to a redistribution of absorbed energy, increasing the fraction of energy emitted as fluorescence or lost as heat (Fig. 1).

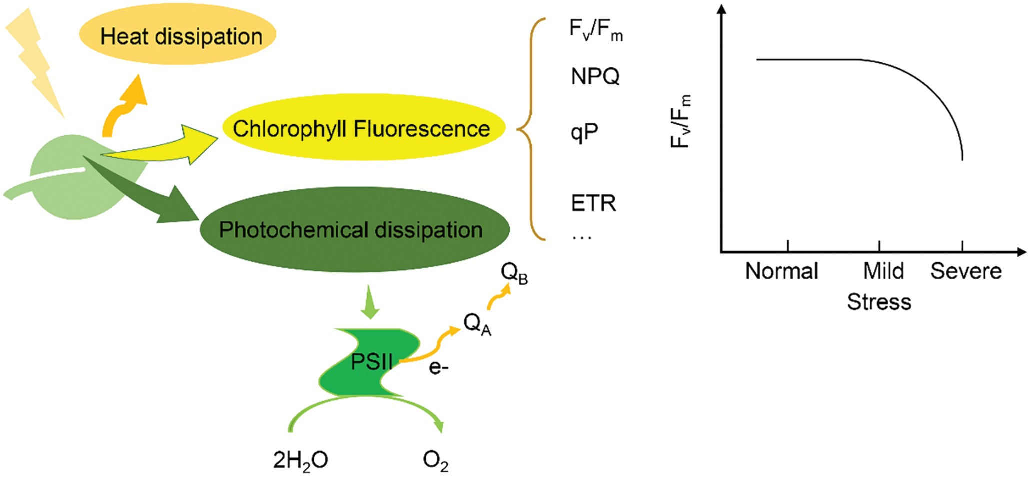

**Figure 1:** The generation principle and characteristics of chlorophyll fluorescence

When light is absorbed by chlorophyll pigments in plant leaves, the excitation energy is distributed among three main pathways: (1) dissipation as heat via non-photochemical quenching, (2) re-emission as chlorophyll fluorescence, and (3) utilization in photochemical reactions through PSII to drive photosynthesis. Within PSII, light energy excites the reaction center chlorophyll (P680), forming an excited state (P680*), which initiates charge separation and electron transfer through the electron transport chain, beginning with the primary electron acceptor $Q_A$ and continuing toward plastoquinone (PQ). Electrons lost from P680 are replenished by the oxygen-evolving complex (OEC), which splits water and releases oxygen. These photochemical processes are closely associated with chlorophyll fluorescence parameters—minimum fluorescence ($F_o$), maximum fluorescence ($F_m$), and variable fluorescence ($F_v = F_m - F_o$). Under environmental stress, the distribution of absorbed light energy shifts, altering fluorescence dynamics. Notably, the $F_v/F_m$ ratio remains relatively stable under mild stress but shows a marked decline under severe stress, making it a sensitive indicator of PSII photoinhibition and overall plant physiological status.

Chlorophyll fluorescence parameters, such $F_v/F_m$ and the effective quantum yield ($\Phi_{PSII}$), reflect the efficiency of photosystem II photochemistry and are commonly used to assess the physiological status of plants. These parameters can be obtained using Pulse-Amplitude Modulation (PAM) fluorometers, PAM fluorometers do not directly quantify fluorescence parameters but instead measure the changes in chlorophyll fluorescence intensity under controlled light pulses, which are then used to calculate photosynthetic parameters by separating photochemical and non-photochemical energy dissipation processes [10]. These instruments facilitate the real-time monitoring of PSII function, offering a dynamic view of how plants respond to their environment.

### *2.2 Definition and Interpretation of $F_v$, $F_m$, and the $F_v/F_m$ Ratio*

The maximum quantum yield of PSII, represented as $F_v/F_m$, is calculated from two core fluorescence parameters (Fig. 2):

- $F_o$ (minimum fluorescence): The basal level of fluorescence when all PSII reaction centers are open (in the dark-adapted state).
- $F_m$ (maximum fluorescence): The maximal fluorescence emission when all PSII reaction centers are closed following a saturating light pulse.

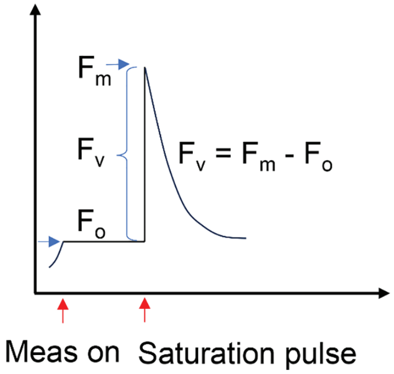

**Figure 2:** Simple illustration of $F_v/F_m$

The variable fluorescence ($F_v$) is defined as the difference between $F_m$ and $F_o$:

$$F_v = F_m - F_o$$

Thus, $F_v/F_m = (F_m - F_o)/F_m$.

Under dark adaptation conditions, the leaf samples were placed for 20–30 min to ensure that the reaction centers of PSII were fully open. The leaf discs were then positioned securely under the measuring probe to ensure firm contact with the sensor. The MINI-PAM first recorded the $F_o$, which is the fluorescence emitted under a weak measuring light. Subsequently, a strong saturating pulse was applied to fully close the PSII reaction centers, and $F_m$ was recorded.

This ratio is a widely accepted indicator of the intrinsic efficiency of PSII photochemistry in dark-adapted leaves. Under optimal conditions, most plant species exhibit an $F_v/F_m$ ratio of approximately 0.83. Deviations from this value are typically associated with stress or photoinhibition [3]. An $F_v/F_m$ value significantly lower than 0.80 may indicate that PSII reaction centers are either damaged or less efficient, which may result from excessive excitation energy, photodamage, or oxidative stress [11].

### *2.3 Factors Affecting $F_v/F_m$ Values in Plants*

A wide range of factors can influence $F_v/F_m$ values, making their interpretation context-dependent. Drought, salinity, heat, cold, and nutrient deficiencies are all known to reduce $F_v/F_m$ values. For instance, water stress impairs PSII activity by causing thylakoid membrane disorganization and an increase in energy dissipation as heat [12]. Pathogenic infections can also impair electron transport and energy conversion in PSII, leading to altered chlorophyll fluorescence dynamics [13,14]. However, specific effects depend on pathogen type and host defense mechanisms. Inter-specific and intra-specific differences exist in baseline $F_v/F_m$ values and in their stress responses. Some species have naturally higher resilience and efficient repair mechanisms for photodamaged PSII centers [15]. Young leaves often have higher $F_v/F_m$ ratios compared to senescing tissues [16]. Chloroplast development, pigment composition, and structural integrity of thylakoid membranes all evolve with plant age, affecting fluorescence parameters [17].

### *2.4 Integrated Models of PSII Energy Partitioning and $F_v/F_m$ Prediction*

Although conceptually distinct, the components of PSII energy partitioning interact dynamically in response to environmental cues. Under high light or stress, NPQ mechanisms (particularly xanthophyll cycling and proton gradient formation) increase, reducing $F_v/F_m$ to protect the PSII complex from over-excitation [10]. One commonly used conceptual framework is the “energy flux model,” which partitions the energy budget in PSII across specific fluxes—such as energy absorption (ABS), trapping (TR), electron transport (ET), and dissipation (DI). Each can be measured per reaction center or per cross-section, facilitating a detailed energy balance under different physiological states [18]. Another evolving model involves machine learning-based prediction of $F_v/F_m$ under fluctuating field conditions, where factors such as light, temperature, and humidity rapidly change. For example, Xia et al. [9] developed a Least-Squares Support Vector Machine (LSSVM) model that estimates $F_v/F_m$ without the need for dark adaptation, opening avenues for high-throughput phenotyping (Fig. 3). They selected environmental variables such as light intensity, temperature and humidity, as well as the spectral characteristics of leaf reflection as input variables. During the data collection process, multi-parameter synchronous measurements were conducted on various plants at different time periods and growth stages to obtain a large amount of sample data for model training and validation. In addition, the specific steps for model training and validation were described. Then, the validation set data was used to evaluate the prediction accuracy and generalization ability of the model. The computation time for each test sample was less than 4 ms. Furthermore, the model demonstrated strong predictive performance on the test dataset, with a high correlation coefficient ranging from 0.762 to 0.974, a low Root Mean Squared Error (RMSE) between 0.005 and 0.021, and a Residual Prediction Deviation (RPD) ranging from 1.254 to 4.933.

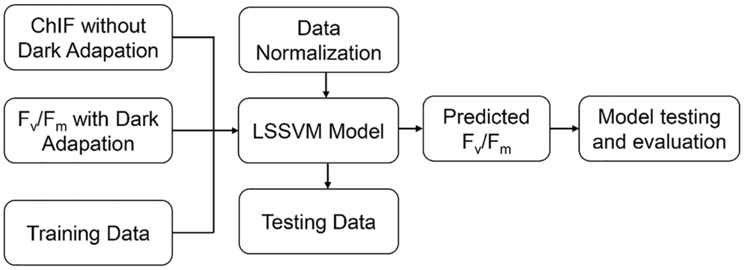

**Figure 3:** The development process of an LSSVM model

The development process of an LSSVM model used to predict $F_v/F_m$ values from chlorophyll fluorescence data collected without dark adaptation. The input and target data are first normalized, then split into training and testing sets. After training, the LSSVM model predicts $F_v/F_m$ values for test samples, and its performance is evaluated using RMSE, Correlation Coefficient (CC), and Residual Predictive Deviation (RPD) to assess accuracy and robustness.

## 3 Methodological Approaches for Measuring $F_v/F_m$

### *3.1 Overview of Standard Protocols and Equipment (e.g., PAM Fluorometry)*

The most widely adopted method for quantifying $F_v/F_m$ is PAM fluorometry, which allows high-sensitivity, non-invasive monitoring of chlorophyll fluorescence parameters in both laboratory and field conditions. The PAM technique employs a series of actinic and saturating light pulses to distinguish between the minimum fluorescence ($F_o$), maximum fluorescence ($F_m$), and hence the variable fluorescence ($F_v$), which forms the basis of the $F_v/F_m$ calculation. A simplified JIP-test, used in commercial systems, focuses specifically on the $F_v/F_m$ ratio to monitor PSII photochemical efficiency under different physiological states. This approach has been integrated into portable platforms and high-throughput imaging systems, such as the MINI-PAM and Imaging-PAM devices, which enable detailed spatial and temporal fluorescence mapping [19,20].

### *3.2 Advances in Imaging Techniques and High-Throughput Phenotyping*

In recent years, chlorophyll fluorescence imaging has revolutionized the phenotyping landscape. Unlike point-based PAM measurements, fluorescence imaging allows spatially resolved data collection from entire leaves or plant canopies. This is particularly useful for visualizing heterogeneities in PSII efficiency and detecting localized stress responses. High-throughput platforms such as GROWSCREEN-FLUORO and automated phenotyping chambers combine RGB imaging with chlorophyll fluorescence analysis to screen large populations of plants efficiently. These systems can evaluate $F_v/F_m$ alongside morphological traits such as leaf area or shape, enabling integrative phenotyping of stress resilience or genetic variability [21,22]. Fluorescence imaging has also been successfully applied in biotic stress studies (Fig. 4). For example, Hupp et al. used high-resolution fluorescence imaging to detect PSII damage caused by *Pseudomonas syringae* in *Arabidopsis*, revealing localized reductions in $F_v/F_m$ even before visible symptoms appeared [23]. Moreover, cutting-edge studies are integrating machine learning and spectral modeling to estimate $F_v/F_m$ without the need for traditional dark adaptation protocols. These computational methods enhance real-time monitoring and have promising applications in remote sensing and field-based decision support systems [24].

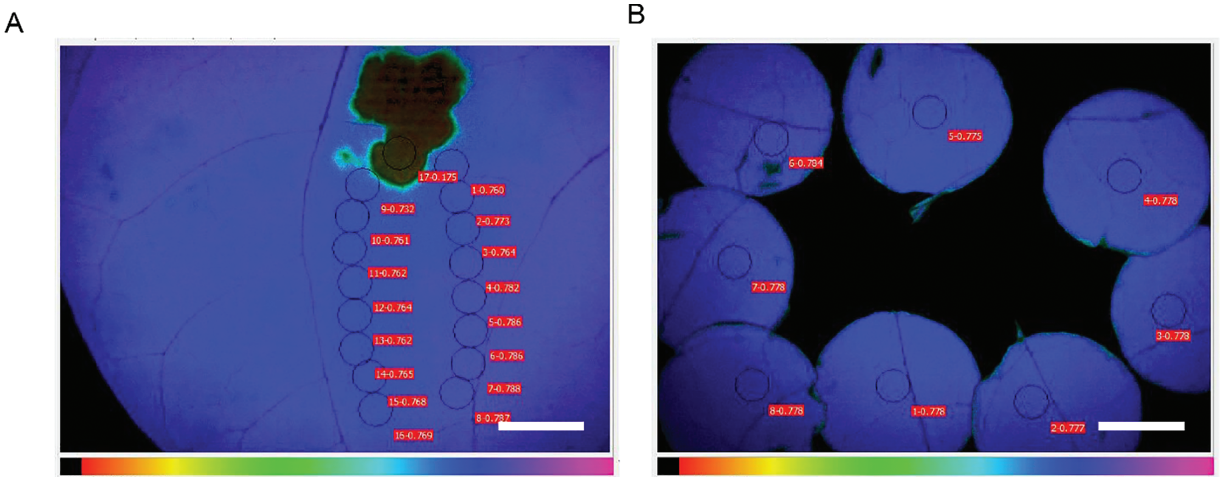

**Figure 4:** Fluorescence imaging of $F_v/F_m$. (**A**), Fluorescence imaging of $F_v/F_m$ was performed using the Imaging-PAM MINI system. The resulting images show the spatial distribution of $F_v/F_m$ values across leaf surfaces following pathogen infection. A reduced $F_v/F_m$ value was observed near the infection site, indicating severe PSII damage. It gradually increases with the increase of distance. indicating that the PSII function is affected to a certain extent or relatively normal. (**B**), Chlorophyll fluorescence measurements were conducted on multiple leaves simultaneously using a chlorophyll fluorescence meter. To ensure uniform sample size, leaf discs were collected using a hole puncher. Measurement points were then selected consistently on each disc to maintain accuracy and comparability across samples. Scale bar = 0.5 cm

### *3.3 Methodological Limitations and Considerations in Field vs. Lab Measurements*

While lab-based $F_v/F_m$ measurements provide precise environmental control and repeatability, field measurements introduce several challenges, including fluctuating light conditions, variable ambient temperatures, and wind-induced leaf movement—all of which can affect fluorescence signals. To mitigate these issues, researchers often use leaf clips for dark adaptation and shielding devices to stabilize measurements. Another complication arises from diurnal variation; $F_v/F_m$ values can fluctuate significantly over the course of a day due to reversible photoinhibition [25,26]. Therefore, standardizing the time of day for measurements is essential for field studies. Additionally, stress imposition (e.g., drought) may manifest at different spatial scales, leading to potential underestimation or overestimation of photoinhibition when only specific leaf regions are measured [27].

### *3.4 Evaluation of Data Quality and Reproducibility in Recent Studies*

The reliability and reproducibility of $F_v/F_m$ data have been central concerns in phenotyping research. A study by Baker et al. demonstrated that fluorescence-based indicators, including $F_v/F_m$, exhibit high repeatability and correlate well with biomass and yield components in crops [28]. However, reproducibility can vary depending on the instrumentation, operator handling, environmental conditions, and calibration routines. To address these challenges, Bates (2020) emphasized the importance of integrating automated control systems and Standard Operating Procedures (SOPs) to minimize measurement artifacts and inter-experimental variability [29]. Furthermore, Kovář et al. validated the consistency of fluorescence-based imaging in drought-stressed tomato plants, confirming that well-calibrated digital imaging systems can produce reliable, non-destructive phenotypic data over multiple time points [30]. Advancements in PAM fluorometry, imaging technologies, and automation have substantially improved the sensitivity, scalability, and utility of $F_v/F_m$ measurements. Tschiersch et al. (2017) integrated kinetic PAM fluorometry into automated phenotyping platforms, enabling reproducible $F_v/F_m$ measurements on up to ~180 large plants per hour [31]. Integration with other imaging modalities has further improved data fidelity. A Plant Methods (2024) study fused RGB, hyperspectral, and chlorophyll fluorescence imaging, achieving over 98% pixel-level alignment that reduces measurement noise and enhances spatial resolution for stress detection [32]. Controlled-environment throughput is enhanced by custom fluorescence chambers capable of dynamic gas–light protocols, enabling simultaneous measurement of over 500 samples daily—enhancing both experimental control and data quality in kinetic $F_v/F_m$ assays [33]. Nevertheless, ensuring data quality—particularly under variable field conditions—requires rigorous standardization and attention to methodological nuance. The increasing integration of AI tools and modeling approaches holds promise for overcoming many existing limitations and extend the applicability of $F_v/F_m$ as a robust phenotyping metric [5].

## 4 Recent Advances in $F_v/F_m$ Research

### *4.1 Abiotic Stress Responses and Applications*

The $F_v/F_m$ parameter has been extensively applied in evaluating plant responses to a variety of abiotic stresses, including drought, heat, cold, salinity, and nutrient deficiencies. In these contexts, $F_v/F_m$ acts as a sensitive indicator of photoinhibition and the decline in PSII efficiency under adverse conditions. For instance, recent studies have demonstrated that drought conditions significantly lower $F_v/F_m$ values due to impaired electron transport and increased oxidative stress. A study on soybean demonstrated that moderate to severe drought led to significant reductions in both $F_v/F_m$ (up to 63.9%) and $\Phi_{PSII}$, along with increased NPQ and reactive oxygen species accumulation, reflecting photoinhibition and oxidative damage to the photosynthetic apparatus [34]. In wheat and chickpea, drought-induced decreases in $F_v/F_m$ correlated with reduced biomass and yield, making this parameter valuable for early stress detection and crop selection [35]. $F_v/F_m$ holds particular promise for drought mapping, especially when integrated into remote sensing frameworks. Under water-limited conditions, declines in $F_v/F_m$ often precede visible wilting, making it a physiologically grounded warning signal of drought stress. Although traditional vegetation indices such as NDVI and PRI have been widely used in drought monitoring, they suffer from limitations, including saturation under high biomass, sensitivity to soil background, and reduced reliability in heterogeneous canopies. Moreover, NDVI often reaches a plateau under dense vegetation, limiting its sensitivity to physiological stress, while PRI is highly sensitive to canopy structure and illumination conditions. In contrast, $F_v/F_m$ reflects intrinsic photochemical efficiency and is less affected by structural and background noise, making it more robust in diverse environments. For practical drought assessment, integrating $F_v/F_m$ with other parameters—such as leaf water content, thermal imagery, and soil moisture data—can improve both spatial and temporal sensitivity. As demonstrated by Zielinska et al. (2021), multi-source data fusion—combining optical, thermal, and microwave remote sensing—enables more reliable, near-real-time assessment of drought impacts on vegetation [36]. Incorporating $F_v/F_m$ into such models could overcome spectral limitations and enhance physiological relevance. However, for $F_v/F_m$ to be incorporated into operational drought monitoring systems, it is essential to determine whether it can be autonomously measured and scaled using remote sensing or proximal sensing platforms. High temperatures also cause irreversible damage to the D1 protein in PSII, reflected by sustained declines in $F_v/F_m$. A study by Bhardwaj et al. [37] employed $F_v/F_m$ phenotyping alongside heat injury indices to screen for thermotolerant crop genotypes, highlighting its utility in breeding programs targeting heat stress resilience. Salinity stress has similarly been associated with photodamage in crops like chickpea and rice. Recent research showed that genotypic tolerance to salt could be rapidly assessed using imaging systems that quantify $F_v/F_m$, aiding in high-throughput climate-smart breeding efforts [38,39]. Notably, spatial heterogeneity in $F_v/F_m$ under these stresses has been visualized using false-color fluorescence imaging, facilitating detection of localized stress zones and enabling site-specific interventions [40].

### *4.2 Biotic Stress and Pathogen Interactions and Applications*

$F_v/F_m$ also plays a critical role in detecting plant responses to biotic stress, including infections caused by fungi, bacteria, and viruses. Pathogen attacks disrupt PSII electron transport, resulting in decreased $F_v/F_m$ in infected tissues—often before visible symptoms emerge. For example, Cséfalvay et al. demonstrated that grapevine leaves infected by *Plasmopara viticola* exhibited a sharp decline in $F_v/F_m$, allowing early detection via chlorophyll fluorescence imaging [41]. This precision makes $F_v/F_m$ a valuable non-invasive marker for plant immunity screening and disease resistance mapping. Additionally, high-resolution fluorescence phenotyping has been used to differentiate between resistant and susceptible genotypes by analyzing their recovery of $F_v/F_m$ following pathogen exposure. Such applications are gaining traction in integrated disease management and smart agriculture.

### *4.3 $F_v/F_m$ in Ecosystem Productivity and Carbon Balance Modeling*

$F_v/F_m$ is increasingly recognized not only as a diagnostic indicator of stress but also as a critical proxy for quantifying photosynthetic efficiency in carbon cycle studies. As a measure of the maximum quantum efficiency of Photosystem II (PSII), $F_v/F_m$ is directly linked to the capacity of vegetation to convert absorbed light into chemical energy [42], making it highly relevant to estimates of carbon assimilation such as Gross Primary Production (GPP) and Net Ecosystem Exchange (NEE). Carbon fluxes such as GPP and NEE are typically measured using eddy covariance towers or gas exchange chambers. When modeled alongside $F_v/F_m$, studies have shown that declines in $F_v/F_m$ correspond closely with reductions in net carbon uptake, particularly in drought- and heat-stressed ecosystems. For instance, in Mediterranean oak woodlands, reduced $F_v/F_m$ values under summer drought conditions aligned temporally with decreased flux-tower GPP readings. Integrating $F_v/F_m$ into Light Use Efficiency (LUE) models enhances the ability to simulate GPP under varying environmental conditions, particularly in ecosystems experiencing stress from drought, temperature extremes, or nutrient limitations. Reductions in $F_v/F_m$ typically precede or coincide with declines in carbon uptake, making it a useful early-warning signal. Remote sensing platforms equipped with solar-induced chlorophyll fluorescence (SIF) instruments have increasingly leveraged $F_v/F_m$ -related signals to improve large-scale estimates of GPP and NEE [43–45]. For example, Nichol et al. demonstrated that canopy-level chlorophyll fluorescence, including $F_v/F_m$, is strongly correlated with ecosystem GPP across various landscapes [46]. Similarly, Zarco-Tejada et al. support this coupling in diverse climatic zones, offering compelling evidence that $F_v/F_m$ variations can serve as warning indicators of photosynthetic downregulation prior to measurable changes in carbon fluxes [47]. These relationships are particularly critical in climate-sensitive ecosystems such as boreal forests, savannas, and peatlands, where early stress detection can guide adaptive land management. Modeling $F_v/F_m$ provides not only a mechanistic understanding of plant stress responses but also a scalable tool for interpreting and predicting ecosystem carbon dynamics. This integration bridges physiological measurements with ecosystem-scale carbon monitoring, offering valuable insights for climate-resilient land management and predictive Earth system modeling.

### 4.4 Genotype and Species-Level Variability and Applications

In recent years, $F_v/F_m$ has become a cornerstone trait for quantifying intraspecific and interspecific variability in PSII efficiency under environmental stress. Differences in $F_v/F_m$ among genotypes offer insight into their relative tolerance and adaptability. In studies on heat-tolerant wheat and rice, genotypes with higher $F_v/F_m$ values under stress maintained higher biomass and yield, confirming the value of $F_v/F_m$ in selective breeding pipelines [48,49] used genotype-by-yield × trait biplot analysis to demonstrate that $F_v/F_m$ can effectively rank genotypic responses to salinity stress when coupled with physiological and spectral traits [50]. This genotype-level application has expanded to wild species and perennial crops as well, offering a scalable tool for screening genetic resources for climate resilience across plant biodiversity.

### 4.5 Remote Sensing and Precision Agriculture Applications

Technological advances have enabled the integration of $F_v/F_m$ into remote sensing platforms, particularly unmanned aerial vehicles (UAVs) and satellite equipped with multispectral and fluorescence imaging sensors. These systems now facilitate rapid, non-invasive, and large-scale crop monitoring [51]. UAV-based imaging enables quantification of $F_v/F_m$ across entire fields, identifying spatial heterogeneity in photosynthetic performance and detecting zones of abiotic or biotic stress. Wu et al. demonstrated the integration of UAV-derived $F_v/F_m$ with environmental variables to track wheat genotypes in heterogeneous landscapes [5]. Furthermore, Anshori et al. [52] highlighted UAV imaging as part of the "Agri 4.0" toolbox for real-time phenotyping, with $F_v/F_m$ included as a key metric for precision stress diagnostics and crop decision-making. Notably, strong correlations have been observed between UAV-derived $F_v/F_m$ data and physiological stress indices, validating its use in real-time agronomic management and early stress alert systems [53].

Beyond UAVs, satellite remote sensing has emerged as a powerful platform for estimating $F_v/F_m$ at broader spatial and temporal scales. Recent work by Bartold et al. (2024) modeled satellite-derived $F_v/F_m$ using machine learning algorithms trained on satellite imagery and *in-situ* reference data, achieving accurate estimates of plant stress in inaccessible wetland areas [54]. These advances suggest that satellite-based monitoring of PSII efficiency can complement ground measurements and UAV data in large-scale plant health assessments. Importantly, feature importance analysis in these models reveals that spectral bands in the red (~680–690 nm) and far-red (~730–740 nm) regions—closely associated with solar-induced chlorophyll fluorescence (SIF)—are among the most predictive variables for $F_v/F_m$ estimation. Techniques such as permutation feature importance in random forest models and SHAP (SHapley Additive exPlanations) have been used to confirm that these spectral regions consistently rank among the top predictors. Their high sensitivity to changes in chlorophyll content, energy dissipation, and photoinhibition makes them superior to broadband indices in detecting early physiological stress. Red and far-red bands contribute complementary physiological information: while the red fluorescence peak (~683 nm) reflects photosystem II activity and often saturates under high stress, the far-red peak (~736 nm) remains responsive, enabling more reliable stress detection across a wider range of conditions [55]. The red/far-red fluorescence ratio, derived from these peaks, is a sensitive indicator of chlorophyll degradation and can distinguish between different stress types, including drought, nutrient deficiency, and disease [56].

### 4.6 Classical Statistical Methods for $F_v/F_m$ Estimation

Prior to the widespread adoption of machine learning, classical regression models played a foundational role in estimating $F_v/F_m$ from remotely sensed and *in-situ* data. A foundational reference highlights the use of linear regression to model $F_v/F_m$ before the widespread adoption of machine learning. In this study, photon yields were calculated by linear regression from chlorophyll fluorescence data, including $F_v/F_m$, indicating early statistical modeling of this parameter in plant stress research [57]. Additionally, modern works continue to support the relevance of $F_v/F_m$ as a stress indicator in crops like sugar beet and maize. For example, recent research has identified $F_v/F_m$ as a drought stress marker in maize and sugar beet [11,57,58], and has analyzed photosynthetic responses under heat stress conditions [59]. This underscores that the application of linear regression to $F_v/F_m$ analysis has a well-established precedent in stress physiology, particularly in the era preceding modern machine learning tools.

In summary, the past decade has seen $F_v/F_m$ evolve from a lab-based physiological marker to a field-deployable, high-resolution trait central to modern phenotyping and precision agriculture. Its applications span stress physiology, disease detection, genetic selection, and remote monitoring—positioning it as a pivotal tool for climate-resilient and sustainable plant science.

## 5 Relationships Between $F_v/F_m$ and Other Physiological or Biochemical Parameters

### 5.1 Correlation with Photosynthetic Activity and Gas Exchange

The chlorophyll fluorescence parameter $F_v/F_m$ does not operate in isolation. Instead, it is closely linked to a suite of physiological processes and biochemical responses in plants. $F_v/F_m$ exhibits a strong and often linear relationship with net photosynthetic rate ($P_n$) and stomatal conductance ($G_s$) under stress conditions. These parameters co-decline under environmental pressures such as heat, drought, and salinity, reflecting reduced carbon assimilation efficiency due to compromised PSII activity. For example, in drought-stressed wheat, declines in $F_v/F_m$ were accompanied by reduced $P_n$ and $G_s$, underscoring the integral role of PSII efficiency in whole-plant gas exchange [60]. Similarly, studies in tomato have shown that $F_v/F_m$ tightly correlated with changes in electron transport rate and stomatal behavior, particularly under exogenous melatonin treatment [61]. This intervention improved $F_v/F_m$ and simultaneously enhanced stomatal aperture and photosynthetic electron flux, suggesting that PSII recovery under stress can restore gas exchange performance. In *Robinia pseudoacacia*, elevated $CO_2$ concentrations caused a decrease in $F_v/F_m$, which was associated with altered stomatal regulation and leaf proteome profiles. This highlights how external carbon flux can also affect photochemical efficiency through stomatal and metabolic interactions [62].

### 5.2 $F_v/F_m$ and ROS Production

ROS such as hydrogen peroxide ($H_2O_2$), superoxide ($O_2^-$), and hydroxyl radicals (•OH) are natural metabolic byproducts of photosynthesis and respiration. While low levels serve as signaling molecules in plant growth and development, high ROS concentrations induce oxidative stress and cellular toxicity, damaging lipids, proteins, and DNA. Abiotic stress frequently triggers ROS accumulation such $H_2O_2$, which can damage thylakoid membranes and impair PSII function [63]. This oxidative burden results in reduced $F_v/F_m$ values due to photoinhibition and decreased electron transport. In rice under drought stress, data revealed elevated antioxidant enzyme activity and ROS scavenging pathways in genotypes that maintained higher $F_v/F_m$ values—demonstrating a physiological link between ROS detoxification and photoprotection [64].

Furthermore, studies on *Stylosanthes* exposed to manganese toxicity confirmed that ROS accumulation was negatively associated with $F_v/F_m$. Plants with impaired ROS defense mechanisms exhibited greater photoinhibition, reinforcing the role of oxidative stress in modulating fluorescence responses [65]. According to Akhter et al. (2021). and Lee et al. (2013), a notable reduction in $F_v/F_m$ was observed in rice and barley under salt stress, concurrent with increased ROS accumulation [66,67]. The study highlighted how PSII damage, due to ionic toxicity and osmotic stress, reduced photosynthetic efficiency, resulting in excessive excitation energy and ROS generation as a byproduct of impaired electron transport chains.

### 5.3 Integration with Transcriptomics and Proteomics

Omics approaches have greatly enriched our understanding of $F_v/F_m$ as a systems-level marker. Transcriptomic studies indicate that genes involved in photosynthesis, photoprotection, and redox regulation are closely co-expressed with $F_v/F_m$ responses. On the proteomics front, declines in $F_v/F_m$ are associated with reductions in Rubisco content, PSII core proteins, and ATP synthase complexes. A comprehensive study in *Robinia* revealed that reductions in $F_v/F_m$ under high $CO_2$ were accompanied by changes in proteins involved in energy metabolism and photosynthetic repair [62]. In another case, iTRAQ-based proteomic analysis in arsenic-stressed *Brassica napus* showed strong correlations between reduced $F_v/F_m$ and downregulation of chloroplast proteins associated with electron transport and photoprotection [68]. Additionally, Rafiq & Fatima highlighted how transcriptomics and proteomics converge to reveal ROS-related gene networks that regulate both photosynthetic capacity and $F_v/F_m$ dynamics under oxidative stress [69].

## 6 Research Gaps and Controversies

Despite its broad application as a key indicator of PSII efficiency, the $F_v/F_m$ parameter has several limitations and interpretational ambiguities. Its sensitivity is influenced by the type and severity of stress, plant species, and environmental conditions. For example, under moderate drought stress, $F_v/F_m$ may remain unchanged, whereas other parameters like $\Phi_{PSII}$ or NPQ might show significant variations [70]. Moreover, factors such as measurement timing and methodology can influence $F_v/F_m$ readings, necessitating standardized protocols for accurate comparisons. Significant declines in $F_v/F_m$ typically occur only under severe stress, such as prolonged drought or heat, resulting from photoinhibition or PSII damage [60,71]. Additionally, considerable interspecific variability exists in baseline $F_v/F_m$ values, influenced by leaf anatomy, chloroplast distribution, and PSII stoichiometry. This variability, compounded by differences in measurement protocols—such as actinic light intensity, dark adaptation duration, and sampling geometry—complicates the application of $F_v/F_m$ as a universal stress diagnostic and calls for species-specific calibration. In terms of spatial and temporal resolution, standard PAM fluorometry captures only point-based data, potentially overlooking spatial heterogeneity in stress responses across leaves or canopies. While chlorophyll fluorescence imaging (CFI) improves spatial resolution, its field application is limited by equipment limitations and ambient light interference. UAV-based remote sensing holds promise for large-scale assessment, although limited spatial resolution can mask localized stress zones. Furthermore, $F_v/F_m$ is subject to diurnal fluctuations and environmental noise, requiring strict measurement standardization to ensure repeatability.

To address these limitations, future research should prioritize the development of standardized, species-adaptive protocols, the integration of $F_v/F_m$ with complementary indicators such as NPQ, as well as electron transport rate (ETR), and antioxidant enzyme activity, and the advancement of imaging technologies capable of high-resolution, real-time diagnostics. In addition, incorporating parameters such as $Q_A$ reoxidation kinetics, fluorescence lifetime, gas exchange rates, and net photosynthetic assimilation can provide a more comprehensive and quantitative assessment of plant stress responses. Such approaches will be critical for improving the interpretability and predictive power of $F_v/F_m$ in ecological monitoring and stress physiology.

## 7 Connection to Current and Future Research

As a robust indicator of PSII efficiency, $F_v/F_m$ has been increasingly integrated into high-throughput phenotyping and breeding platforms, aiding in early stress detection and trait selection under abiotic challenges such as drought and salinity. When combined with omics data, $F_v/F_m$ enhances genotype-to-phenotype resolution. Recent advancements in UAV and satellite-based imaging have enabled spatial monitoring of crop health and vegetation performance [54]. The multispectral and RGB images of unmanned aerial vehicles based on the LSSVM model are expected to promote widespread adoption of chlorophyll fluorescence detection in agriculture, ecological monitoring of algae in lakes and oceans. Concurrently, AI and machine learning approaches have enabled real-time stress prediction without dark adaptation, offering scalable solutions for precision agriculture. Beyond crops, $F_v/F_m$ is now applied in ecosystem research across aquatic, arid, and high-altitude environments to assess resilience and productivity under climate stress. However, challenges remain, including inconsistent protocols, species-specific variability, and limited temporal resolution. Addressing these through standardization and sensor innovation will further enhance $F_v/F_m$'s reliability. Moving forward, dynamic modeling and sensor fusion will transform $F_v/F_m$ from a diagnostic to a predictive tool. In sum, $F_v/F_m$ now serves as a cornerstone of climate-smart plant science, uniting digital agriculture, ecological forecasting, and stress physiology in the face of global environmental change.

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