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REVIEW

Recent advances in plant stress analysis using chlorophyll *a* fluorescence

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Abstract

Chlorophyll fluorescence (ChlF), a sensitive, real-time, and nondestructive indicator of photosynthesis, enables noninvasive elucidation of the complex physiological and biochemical processes of plants. It plays a unique and important role in plant research, ecological evaluation, and agriculture. To provide a holistic picture of research on ChlF applications over the past decade, a knowledge map was first conducted, which revealed six major areas of ChlF applications in plant stress evaluation and reduction, including drought stress, temperature stress, salt stress, water stress, toxicity stress, and nitrogen stress. This work then systematically summarized the literature in each of the six areas. Finally, we examined practical application bottlenecks and outlined key challenges and frontiers in future ChlF research.

Keywords: chlorophyll fluorescence; knowledge mapping; noninvasive detection; photosynthesis; plant stresses.

Introduction

With increasingly severe global ecological challenges, the fundamental role of plants in human survival and development has become ever more prominent. Plants not only provide the core energy and material source for the Earth's life systems through photosynthesis but also serve as crucial natural forces mitigating rising atmospheric

Highlights

- Major research areas were identified through a systematic bibliometric analysis
- Recent advances in chlorophyll fluorescence in plant stress studies were summarized
- Future research directions of chlorophyll fluorescence applications were discussed

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Abbreviations: ABS/RC – absorption per reaction center; BR – brassinolide; CFI – chlorophyll fluorescence imaging; ChlF – chlorophyll *a* fluorescence; DI_0/RC – dissipation per reaction center; ET_0/ABS – quantum yield of electron transport; ET_0/CS – electron transport energy flux; ET_0/RC – electron transport per reaction center; ETR – electron transport efficiency; F_0 – minimal chlorophyll *a* fluorescence in the dark-adapted state; F_m – maximal chlorophyll *a* fluorescence in the dark-adapted state; F_m/F_0 – electron transport through photosystem II; F_V – variable fluorescence in the dark-adapted state; F_V/F_0 – quantum efficiency of photosystem II; F_V/F_m – maximum photochemical quantum yield of photosystem II in the dark-adapted state; F_V'/F_m' – the efficiency of excitation energy capture by open PSII reaction centers; ML – machine learning; NDVI – the normalized difference vegetation index; NPQ – nonphotochemical quenching coefficient; OJIP – chlorophyll *a* fluorescence induction kinetics curve; PCA – principal component analysis; PI – performance index, which indicates the internal force of the sample to resist constraints from outside; PI_{abs} – performance index on absorption basis; PRI – the photochemical reflectance index; q_P – the photochemical quenching coefficient; QTL – quantitative trait locus; QY – stable maximum quantum yield; RC/ABS – the number of photosynthetically active reaction centers per unit of absorbed light energy; ROS – reactive oxygen species; RWC – relative water content; $Y_{(II)}$ – actual photochemical quantum yield.

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CO_2 concentrations. However, escalating environmental stresses, such as climate change and pollution, are subjecting plants to unprecedented physiological pressures (Nawaz *et al.* 2023). Consequently, accurate and timely assessment of plant physiological health, particularly the responses and adaptations of their photosynthetic functions to environmental changes, is crucial for understanding ecological shifts and ensuring sustainable agricultural development. Unfortunately, conventional lab-based methods for evaluating plant physiology are often destructive, time-consuming, and difficult to apply for rapid, large-scale dynamic monitoring. Technologies based on chlorophyll *a* fluorescence (ChlF) have been essential tools for nondestructive, fast, and sensitive probing of plant photosynthetic functions, which is critical in plant physiological and ecological research (Baker 2008, Kalaji *et al.* 2016).

The powerful measurement capabilities of ChlF-based technologies stem from the intrinsic connection of ChlF to the core processes of plant photosynthesis, particularly photosystem II (PSII) (Maxwell and Johnson 2000, Harbinson 2013, Murchie and Lawson 2013). When chlorophyll molecules absorb light energy, the energy is dissipated primarily through three pathways: driving photochemical reactions, dissipated as heat, and emitted as fluorescence (Guo and Tan 2014). Crucially, ChlF yield exhibits a coupling relationship with both photochemical efficiency and heat dissipation. Therefore, by measuring ChlF signal characteristics [such as minimum fluorescence in dark (F_0), maximum fluorescence in dark (F_m), maximum photochemical efficiency in dark (F_V/F_m), actual photochemical quantum yield ($Y_{(II)}$), and nonphotochemical quenching (NPQ)], it is possible to obtain key physiological information concerning the activity of PSII reaction centers, electron transport efficiency (ETR), photoprotective capacity, and responses to environmental stresses nondestructively and in real time (Genty *et al.* 1989).

The measurement process is nondestructive, allowing for repeated, long-term dynamic monitoring of the same plant or leaf. Data acquisition is extremely rapid, down to millisecond sampling intervals, making it suitable for high-throughput screening and capturing transient photosynthetic responses. It is highly sensitive to minute changes in photosynthetic function, often detecting physiological abnormalities before visible damages occur under stress. It allows *in situ* and *in vivo* measurements, providing a more authentic reflection of physiological status (Tseng and Chu 2017, Legendre *et al.* 2021, Moustaka and Moustakas 2023). Since the discovery of the Kautsky effect (Kautsky and Hirsch 1931), and with the maturation and application of Pulse-Amplitude-Modulation (PAM) technology, ChlF has become one of the standard tools in plant physiological and ecological research.

ChlF has been used to detect various aspects of plant physiology and stress (Guo and Tan 2015). The volume of research literature on ChlF has surged in the past 10 years. Over the past decade, significant advances have emerged in ChlF research. There have been several review papers with a focus on different ChlF applications such as assessment of plant physiological status under abiotic stress (Kalaji *et al.* 2016), relationship between gas-exchange parameters and ChlF (Urban *et al.* 2017), principles and applications of transient fluorescence kinetics (Stirbet *et al.* 2018), photoinhibition mechanisms in PSII reaction centers (Guidi *et al.* 2019), applications for indication of stress and photosynthetic performance (Lysenko *et al.* 2022), and applications of ChlF imaging (CFI) in early disease/pest detection (Grishina *et al.* 2024). There has not been a systematic review of the recent advances in ChlF-based analysis of plant stresses. This review, an extension and update of a review our group published a decade ago (Guo and Tan 2015), aims to bridge this gap.

Bibliometric analysis

To gain an overall view of the research landscape and changes since our last review in 2015, we conducted a systematic bibliometric analysis to observe the major research areas of ChlF applications. From the Web of Science (WOS) Core Collection database, we retrieved papers published over the past two decades (2005–2025) on applications of ChlF techniques by using the search query: TS = ('chlorophyll fluorescence' OR 'Chl a fluorescence' OR 'Chlorophyll a fluorescence'). For the most recent decade (1 June 2015 to 1 June 2025), an initial search yielded 17,216 publications. After limiting the discipline category to "Plant Sciences", a core collection of 6,688 publications was identified. Applying the same search criteria and discipline restriction to the preceding decade (1 June 2005 to 1 June 2015) in the field of "Plant Sciences" resulted in a core collection of 3,614 publications. Detailed metadata (including authors, institutions, source journals, countries/regions, citation counts, and references) was extracted from these two comparable core collections. There has been an 85.1% increase in the number of publications in the past decade over the previous decade, indicating a substantial surge in research activity in this field.

Keyword co-occurrence analysis was performed by using the *VOSviewer* software to construct knowledge graphs, and the keyword maps are shown in Fig. 1 and Fig. 2 for the two decades, respectively. Between 2005 and 2015, ChlF technologies were applied in diverse areas, primarily on drought stress, temperature stress, water stress, and salt stress, along with the assessment of plant growth. In the recent decade (2015–2025), while applications of ChlF technologies continued to revolve around these core areas, a distinct shift in research focus was observable compared with the previous decade: the relative proportion of research on water stress declined, research on salt stress increased, and research in areas such as drought stress, high-temperature stress, abiotic stress, and oxidative stress became more linked to plant growth. Based on this analysis and our previous review (Guo and Tan 2015), this review will focus on the following six key areas of ChlF applications: drought stress, temperature stress, salt stress, water stress, toxicity stress, and nitrogen stress.

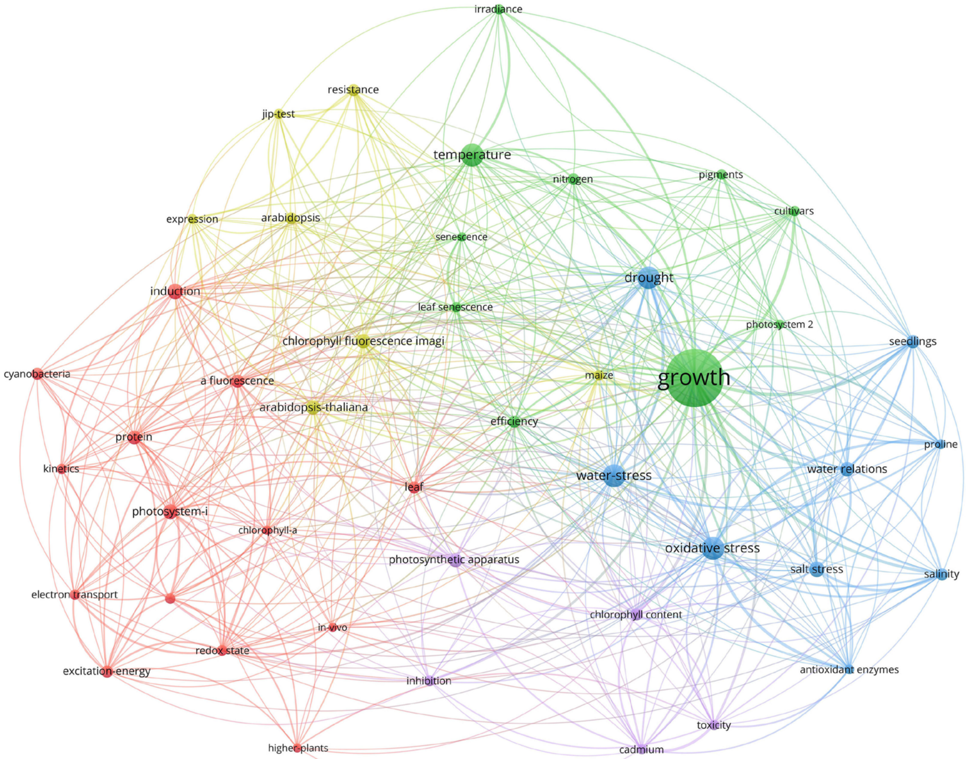

Fig. 1. Keyword clustering related to applications of ChlF from 2005 to 2015.

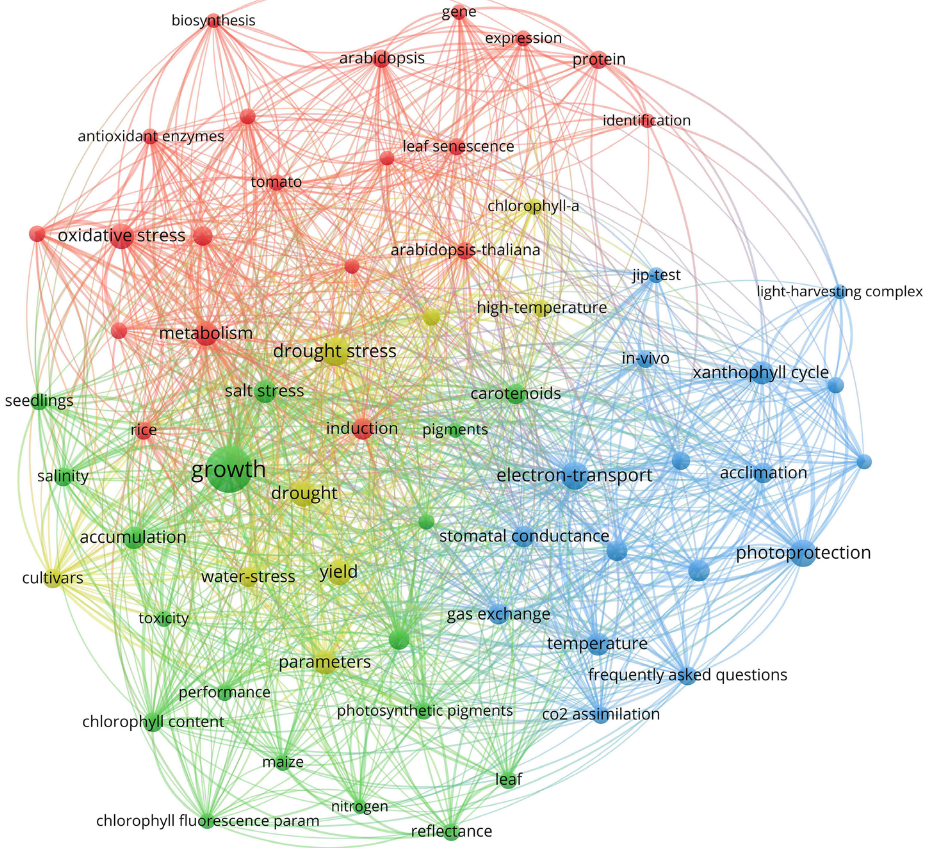

Fig. 2. Keyword clustering related to applications of ChlF from 2015 to 2025.

Recent advances in applications of chlorophyll *a* fluorescence

Drought stress

Drought stress induces stomatal closure and photochemical impairment in plants, thereby altering energy dissipation pathways of PSII. As a result, ChlF parameters can sensitively indicate the degree of damage to the photosynthetic apparatus and reflect photoacclimation strategies of crops (Enyew *et al.* 2022). A summary of recent ChlF applications in sensing drought stress is given in Table 1.

Numerous studies consistently confirm the high sensitivity and reliability of a suite of ChlF parameters to detect drought stress. F_V/F_m and PI_{abs} are established as core diagnostic parameters. These parameters exhibit significant declines under water deficit across various plant species, including wheat, wild barley, and mung bean, and this decrease is directly correlated with ultimate grain yield loss (Jedmowski *et al.* 2015, Kalaji *et al.* 2018, Bano *et al.* 2021, Barboričová *et al.* 2022, Sommer *et al.* 2023). These findings not only validate the universality of F_V/F_m and PI_{abs} as indicators of stress intensity but, crucially, they link initial functional disruption of photosynthetic organs directly to final agricultural output, providing a physiological basis for yield prediction.

Traditional fluorescence measurements based on single characteristic values are being augmented by more advanced techniques. CFI technology expands the scope from "point" to "area", enabling detection of spatiotemporal heterogeneity in PSII photochemical efficiency 15 to 30 min before visible drought symptoms appear. The photochemical quenching coefficient (q_P) has been identified as a sensitive indicator of spatial patterns in light energy allocation (Sánchez-Moreiras *et al.* 2020).

In-depth analysis of the OJIP fast fluorescence induction kinetics curve reveals that the entire curve contains substantially more physiological information than any single parameter. Research demonstrates that classifying drought stress levels in rice using the entire OJIP curve achieved an accuracy of 86.7%, significantly outperforming methods relying solely on F_V/F_m (43.9%) or partial induction characteristics (Xia *et al.* 2022). This signifies a paradigm shift from dependence on individual "static" parameters towards interpreting more information-rich "dynamic" physiological fingerprints. The concomitant generation of large datasets has spurred the application of novel data processing methods, such as dimensionality reduction (Xia *et al.* 2025), which, combined with machine learning, is paving the way for nondestructive and intelligent diagnosis of plant stress status (Long and Ma 2022).

More systematic research strategies involve integrating fluorescence parameters with other key physiological indicators. For instance, correlating PI_{abs} with relative water content (RWC) has successfully facilitated the development of an effective method for screening highly drought-tolerant maize genotypes (Badr and Brüggemann 2020). Similarly, combining fluorescence parameters (*e.g.*, PI_{abs}, ET_0/RC) with gas exchange parameters (*e.g.*, stomatal conductance, photosynthetic rate) enables a more comprehensive elucidation of drought tolerance mechanisms in perennial ryegrass (Dąbrowski *et al.* 2019). This multi-parameter approach effectively bridges the knowledge gap between photochemical processes at the PSII level and gas exchange behavior at the leaf level, constructing a more complete picture of the stress response.

The integration of fluorescence parameters with other key physiological indicators reveals the multi-level damage mechanisms induced by drought stress, spanning from reaction center activity to the electron transport chain, thereby providing critical insights for in-depth research into plant stress responses and mitigation mechanisms. It is noteworthy that while F_V/F_m and PI_{abs} demonstrate a universal response to drought stress across multiple crop species, significant differences exist among different species. For instance, C_4 plants (such as maize) typically exhibit a higher photoprotective capacity compared to C_3 plants (such as sunflower), characterized by a smaller decline in fluorescence parameters and faster recovery

Table 1. Summary of recent ChlF applications in sensing drought stress.

Plant	Main responses and changes	Reference
Sunflower	While sunflower PSII fluorescence parameters exhibit significant decreases under stress, drought-tolerant varieties maintain PSII functionality by enhancing glutathione reductase and superoxide dismutase activities.	Killi *et al.* 2020
Scutellaria	Increased F_0, decreased F_V/F_m, and reduced PI_{abs} collectively indicate impaired PSII electron transport; nevertheless, these plants exhibited drought resilience when RWC was kept above a critical threshold.	Meng *et al.* 2016
Mung bean	Both drought-tolerant and sensitive varieties exhibited reductions in PI_{abs} and F_V/F_m, and drought-tolerant cultivars protected PSII structural stability at high RWC.	Bano *et al.* 2021
Arabidopsis	Long-term drought can activate the PSII repair mechanism and reduce the damage of photo suppression, and the F_V/F_m distribution changes from unimodal to bimodal, and the bimodal spacing can be used as a stress evaluation index.	Chen *et al.* 2016, Bresson *et al.* 2015
Wheat	PI_{abs} and F_V/F_m are extremely sensitive to drought and can be used for drought stress detection.	Barboričová *et al.* 2022, Sommer *et al.* 2023

(Killi *et al.* 2020). This species-specific response suggests that stress assessment based on ChlF parameters requires the establishment of species-specific threshold standards, while also providing new perspectives for research on the evolutionary adaptation of crop drought tolerance.

Temperature stress

Low temperature: Cold stress is a key abiotic stress that damages the plant's photosynthetic system. PSII in the photosynthetic chain is the most sensitive component to low temperatures, making it a central target for deciphering plant cold-response mechanisms (Ji *et al.* 2024). ChlF technology provides crucial technical support for noninvasive and sensitive assessment of this process. A summary of recent ChlF applications in sensing low-temperature stress is given in Table 2.

Numerous studies have shown that the photochemical efficiency of PSII often decreases significantly under cold conditions, manifested by the reduction of key fluorescence parameters such as F_V/F_m and F_V/F_0. For instance, in basil seedlings, the sensitivity of F_V/F_0 to low temperature was even higher than that of the commonly used F_V/F_m, reflecting the differential responses of various fluorescence indicators in stress diagnosis (Kalisz *et al.* 2016). Notably, although F_V/F_m is widely used as an indicator of photoinhibition, its sensitivity may be limited under mild or short-term cold stress. Therefore, a multi-parameter analysis is more conducive to a comprehensive assessment of the stress response.

In recent years, the integration of ChlF technology with imaging systems and intelligent algorithms has significantly enhanced its potential for monitoring plant temperature stress. For example, CFI not only enables the visualization of photosynthetic heterogeneity at the leaf level but also, when combined with fluorescence parameters, facilitates the construction of discriminant models for the precise identification of physiological damage induced by low temperature (Lu and Lu 2020). Furthermore, some studies have integrated ChlF data with unsupervised learning, successfully achieving automatic grading of chilling injury in cucumber seedlings. This demonstrates a novel approach combining high-throughput phenotyping with cold tolerance evaluation (Lu *et al.* 2023). This technological integration represents a future direction in plant phenomics, as it encodes expert physiological knowledge into reusable algorithmic models, paving the way for automated and intelligent large-scale screening of breeding materials.

ChlF parameters also exhibit unique values in evaluating crop freezing tolerance. For instance, Hájek *et al.* (2016) found that the photosynthetic response of lichens to freezing temperatures followed an S-shaped curve, with species-specific critical temperature thresholds, broadening our understanding of freeze injury adaptation mechanisms in non-crop plants. In tomato, wild germplasm maintained more stable F_V/F_m values under low temperatures. Combined with OJIP transient analysis, it was further revealed that the electron transfer on the acceptor side of PSII was less impaired, indicating that the stability of fluorescence parameters is closely related to cold tolerance in germplasm (Zhou *et al.* 2018a). Additionally, using multi-parameter analysis methods such as the JIP-test, researchers identified transgenic zoysia grass genotypes with enhanced PSII functional stability under low temperatures, providing reliable physiological markers for cold-tolerance breeding (Gururani *et al.* 2015, Stirbet *et al.* 2018).

Notably, ChlF technology, particularly the JIP-test based on the OJIP transient, provides comprehensive energy pipeline information ranging from light energy absorption to electron transport. The integrated parameters, such as PI_{abs}, which incorporate multidimensional information including reaction center density, energy flux, and electron transport efficiency, generally demonstrate greater robustness and sensitivity in assessing stress intensity compared to single parameters like F_V/F_m. However, we must clearly recognize that ChlF measurements are susceptible to influences from plant pre-acclimation status, leaf developmental stage, and transient environmental fluctuations. This necessitates extremely strict experimental standardization protocols; otherwise, the universality of conclusions will be challenged.

High temperature: Thermal stress directly impairs photosynthesis by disrupting the structure of PSII, inhibiting electron transport, and triggering a burst of reactive oxygen species (Wang *et al.* 2025a). ChlF technology captures dynamic changes in parameters such as F_V/F_m, PI (performance index), and OJIP curves, thereby translating these invisible microscale damage processes into quantifiable phenotypic data and providing a critical window for analyzing plant thermal stress (Mathur *et al.*

Table 2. Summary of ChlF applications in sensing low-temperature stress.

Plant	Main responses and changes	Reference
Basil seedlings	The sensitivity of F_V/F_0 to low temperature was higher than that of F_V/F_m, reflecting the differential responses of various fluorescence indicators in stress diagnosis.	Kalisz *et al.* 2016
Lichens	The photosynthetic response to freezing temperatures followed an S-shaped curve, with species-specific critical temperature thresholds.	Hájek *et al.* 2016
Tomato	Wild germplasm maintained more stable F_V/F_m values under low temperature, and OJIP transient analysis revealed less impairment of electron transfer on the acceptor side of PSII.	Zhou *et al.* 2018b
Zoysia grass	Using multi-parameter analysis methods such as the JIP-test, researchers identified transgenic genotypes with enhanced PSII functional stability under low temperature.	Gururani *et al.* 2015, Stirbet *et al.* 2018

Table 3. Summary of ChlF applications in sensing high-temperature stress.

Plant	Main responses and changes	Reference
Plum tree	The traditional cultivar 'Bistrica' maintained photosynthetic function integrity under high temperature by accumulating protective compounds (proline, phenolics), resulting in higher stability of PI_{abs} and F_V/F_m. The modern cultivar 'Toptaste' showed increased F_0 and DI_0/RC, indicating severe PSII damage.	Viljevac Vuletić *et al.* 2022
Peony	The cultivar 'Huhong' was identified as the most heat-tolerant based on a smaller decrease in PI_{abs} and a lower proportion of damaged DI_0/RC under heat stress.	Li *et al.* 2023
Wheat	OJIP fluorescence transient parameters (*e.g.*, O–J phase) and the PI were effective for screening heat-tolerant genotypes under heat stress, identifying five genotypes with stable photosynthesis.	Jain *et al.* 2018
Wheat	QTL mapping based on ChlF transient parameters revealed that the number of QTLs detected under high-temperature stress was approximately double that under normal conditions.	Azam *et al.* 2015

2023). A summary of recent ChlF applications in sensing high-temperature stress is given in Table 3.

ChlF parameters provide highly sensitive phenotypic indicators for the rapid identification of heat tolerance across different species and even cultivars (Doğru 2021). In plum trees, the traditional cultivar 'Bistrica' demonstrated superior ability to maintain the functional integrity of its photosynthetic apparatus under high temperatures compared to the modern cultivar 'Toptaste'. This was achieved through the accumulation of protective compounds, such as proline and phenolics, resulting in higher stability of PI_{abs} and F_V/F_m. In contrast, 'Toptaste' exhibited a rise in F_0 and an increase in energy dissipation per reaction center (DI_0/RC), clearly indicating severe damage to PSII (Viljevac Vuletić *et al.* 2022). This comparison suggests that modern breeding programs, while selecting for improved agronomic traits, may have inadvertently compromised the inherent stress resistance supported by complex physiological networks in some cultivars.

A similar screening logic proved effective in peony, where the cultivar 'Huhong' was identified as the most heat-tolerant among three tested, based on a smaller decrease in PI_{abs} and a lower proportion of damaged DI_0/RC under heat stress (Li *et al.* 2023). These cases collectively demonstrate that comprehensive fluorescence parameters, particularly the PI, can quantify complex physiological states into comparable data, enabling the early and precise identification of heat tolerance.

A significant advantage of ChlF technology lies in its utility as an efficient phenotyping tool, bridging macro-physiology and micro-genetic mechanisms. In field practice, a study on 20 wheat genotypes under terminal heat stress showed that OJIP fluorescence transient parameters (such as the O–J phase changes) and the PI were effective for screening heat-tolerant genotypes, successfully identifying five genotypes with stable photosynthesis (Jain *et al.* 2018). The value of fluorescence parameters becomes even more pronounced at the genetic level. Quantitative Trait Locus (QTL) mapping based on ChlF transient parameters in wheat seedlings revealed that the number of QTLs detected under high-temperature stress approximately doubled that under normal conditions (Azam *et al.* 2015). This finding is highly insightful, suggesting that heat stress activates genetic networks that remain "silent" under non-stress conditions, providing a new perspective for understanding a plant's latent heat-resistance potential.

In rice, a genome-wide association study (GWAS) integrating F_V/F_m with grains per panicle (GNPP) successfully identified three heat-stress tolerance-related QTLs and candidate genes, leading to the selection of superior heat-tolerant germplasm accessions such as *IRIS 313-8704* and *IRIS 313-11307* (Das *et al.* 2024). This marks the evolution of ChlF technology from a "physiological indicator" to a powerful "gene locator", directly providing a theoretical basis and genetic resources for molecular design breeding.

In natural environments, high temperature often co-occurs with other stress factors. ChlF technology shows unique value in deciphering the synergistic effects of such combined stresses. A study on ginger found that combined heat and waterlogging stress led to the destruction of photosynthetic pigment structures, massive accumulation of reactive oxygen species (ROS), and a significant decline in F_V/F_m and ET_0/RC, causing severe damage to PSII reaction centers and a near-complete loss of photosynthetic function. The damage was far greater than that caused by heat stress alone (Liu *et al.* 2023). This serves as a crucial reminder that, in the context of climate change, conclusions based on single-stress laboratory studies may severely underestimate the physiological dysregulation occurring under field conditions.

Salt stress

Salt stress disrupts plant water and ion homeostasis, inhibits physiological functions, and significantly impedes photosynthesis (Ma *et al.* 2022, Waheed *et al.* 2024). ChlF techniques serve as sensitive tool for detecting and quantifying these alterations, playing an indispensable role in elucidating the mechanisms of stress-induced damage, evaluating mitigation strategies, and screening salt-tolerant germplasm. Recent advances in ChlF applications in salt stress research are summarized in Table 4.

ChlF analysis reveals that the core damage under salt stress to the photosynthetic apparatus is directly manifested as functional disruption of PSII reaction centers. When salt concentration reaches a critical level (*e.g.*, ≥50 mM NaCl), the OJIP fast fluorescence kinetics curve of tomato leaves undergoes significant deformation, with the fluorescence intensities of characteristic points O, J, I, and P generally suppressed. This is followed by a deterioration in a series of JIP-test parameters: a synchronous decline in energy absorption (ABS/RC) and electron transport energy flux (ET_0/CS) clearly depicts the massive inactivation of active reaction centers (RCs) and the simultaneous impairment of energy capture and linear electron transport efficiency (Zushi and Matsuzoe 2017). The collapse of this energy pipeline model precisely reveals the destructive impact of salt stress on the very initial stages of photosynthesis. Furthermore, studies indicate that this inhibition of electron transport is not limited to the donor side of PSII but extends to the acceptor side of PSI, as shown by a significant decrease in PI, marking a systemic downregulation of the entire electron transport chain from PSII to PSI (Loudari *et al.* 2020).

The application of ChlF technology has challenged traditional views on the mechanism of photosynthetic inhibition under salt stress. Stomatal closure has long been considered the primary cause of photosynthetic decline. However, by simultaneously monitoring gas exchange and ChlF, studies have provided compelling evidence that in alfalfa, the decline in photosynthetic rate under salt stress is mainly attributable to reduced PSII activity rather than stomatal limitations (Najar *et al.* 2019). This finding represents a paradigm shift in understanding from "stomatal limitation" to "nonstomatal limitation", highlighting the crucial position of PSII itself as the primary target of salt stress. Based on this mechanistic understanding, emerging technologies are continuously being developed. For instance, integrating CFI with deep learning models (*e.g.*, *ResNet50*) enables precise distinction of salt stress levels in soybean seedlings with an accuracy of up to 98.6% (Deng *et al.* 2024), signifying the transition of this technology from mechanistic research towards high-throughput, intelligent field diagnosis applications.

In exploring pathways for alleviating salt stress, ChlF technology serves as a "rapid indicator" for evaluating the regulatory effects of exogenous substances (Athar *et al.* 2015, Malekzadeh *et al.* 2024). Research confirms that foliar application of glycine betaine significantly improves fluorescence parameters (*e.g.*, F_V/F_m, F_V/F_0) in rapeseed under salt stress, whereas root application is ineffective, clarifying the importance of the application method (Athar *et al.* 2015). Similarly, the combined application of salicylic acid and silicon can effectively reduce F_0 and enhance PI_{abs} in mung bean by regulating ion balance and enhancing oxygen-evolving complex activity, with the protective effect being superior to individual treatments (Ghassemi-Golezani and Lotfi 2015). These results not only provide feasible agronomic mitigation strategies but also establish the practical value of ChlF in quantifying the efficacy of stress-resistance agents.

ChlF technology plays a central role in high-throughput screening for salt-tolerant germplasm. The ability of salt-tolerant crops (*e.g.*, sweet sorghum and grain sorghum) to maintain relatively stable yields in saline-alkali land is physiologically due to their capacity to compensate for the loss of photosynthetic pigments by maintaining RC/ABS and ET_0/RC, which is a typical photosynthetic functional compensation strategy (Sayyad-Amin *et al.* 2016). At the cultivar level, differences in salt tolerance among genotypes can also be clearly distinguished by fluorescence parameters. For example, the perennial ryegrass cultivar 'Roadrunner' exhibited significantly higher stability in PSII fluorescence parameters (F_V/F_m, ETR) under salt stress compared to the 'Nira' cultivar, and principal component analysis (PCA) further confirmed the stronger structural integrity of its PSII electron transport chain (Dąbrowski *et al.* 2016). This indicates that combining core fluorescence parameters with multivariate statistical methods can construct an efficient and reliable system for assessing salt tolerance, greatly accelerating the process of salt-tolerant breeding.

Notably, many damage patterns to the photosynthetic apparatus revealed by ChlF technology exhibit universality across different stress conditions. For example, under drought stress, phenomena such as the deformation of the OJIP curve, a decline in the PI, and the inactivation of reaction centers are also observed, which are highly similar to responses under salt stress. However, significant differences exist among species: C_4 plants (such as maize) can typically maintain relatively high photochemical efficiency under drought through their unique CO_2 concentration mechanism, whereas the PSII of C_3 plants (such as wheat) is more sensitive to water deficit (Stefanov et al. 2022).

Table 4. Summary of ChlF applications in salt stress.

Plant	Stress conditions	Main responses and changes	Reference
Tomato	25, 50, 75, 100 mM NaCl	Exposure to ≥50 mM NaCl directly impairs the PSII reaction center, evidenced by suppressed OJIP transients, inactivation of active RCs, and diminished energy capture with compromised electron transport efficiency.	Zushi and Matsuzoe 2017
Tomato	Hydroponic and salt stress	Salt stress inhibits electron transfer along the PSII→PSI chain, particularly on the PSI acceptor side, resulting in significant suppression of the PI.	Loudari *et al.* 2020
Lucerne	Salt coercion	PSII activity damage rather than stomatal closure is the direct cause of photosynthetic decline. ChlF parameters serve as effective screening indicators for salt-tolerant legumes.	Najar *et al.* 2019

Despite the widespread application of ChlF technology, current research has some limitations. First, most studies are conducted under controlled laboratory conditions, where the stress treatments (*e.g.*, acute, high-concentration salt stress) may differ from the complex field environment of saline-alkali land (*e.g.*, fluctuating salinity levels, interactions with other stress factors), limiting the direct translation of laboratory findings to practical agricultural applications. Second, many studies focus on short-term stress responses, paying insufficient attention to the dynamic adjustments of photosynthetic function exhibited by plants through cumulative processes under long-term, chronic stress. Recent applications of ChlF in evaluating mitigation strategies for salinity stress are summarized in Table 5.

Water stress

Root hypoxia triggered by waterlogging stress rapidly propagates upwards, culminating in a catastrophe for the leaf photosynthetic apparatus (Manghwar *et al.* 2024). Numerous studies have consistently shown that F_V/F_m and $Y_{(II)}$, as core parameters, are highly sensitive to waterlogging stress. A summary of ChlF research in sensing water stress is given in Table 6.

In crops such as peanut and sorghum, prolonged waterlogging duration leads to a significant decline in F_V/F_m and ET_0/CS, directly evidencing damage to PSII reaction centers and inhibition of linear electron transport (Zhang *et al.* 2019, Sharma *et al.* 2022). Notably, this attenuation is not always linear. A study by Wu *et al.* (2024) on peanut during the pod-setting stage captured a dynamic response: short-term waterlogging (3–5 d) even slightly promoted photosynthesis, potentially by temporary improvement in water status, but with sustained stress (7–9 d), inhibitory effects became dominant. This finding critically highlights that when using fluorescence parameters for stress assessment, the "time window" of stress must be strictly considered to avoid misinterpreting short-term acclimation as tolerance.

The great potential of ChlF technology lies in its ability to quantify subtle physiological differences between genotypes. A comparative study by Lin *et al.* (2020) on pumpkin varieties serves as an example. Under waterlogging stress, the waterlogging-tolerant cultivar 'EP' showed significantly smaller declines in F_V/F_m and $Y_{(II)}$ compared to the sensitive cultivar, indicating its PSII core function remained relatively stable under low oxygen. More importantly, the study found that the normalized difference vegetation index (NDVI) and the photochemical reflectance index (PRI) were highly correlated with core fluorescence parameters, opening avenues for high-throughput screening of large populations for waterlogging tolerance using remote sensing.

Similarly, in screening North China landscape shrubs, tolerant species not only maintained stable maximum quantum yield (QY) but also exhibited an active increase in NPQ during later stress stages (Fang *et al.* 2022). This profoundly reveals that true waterlogging tolerance involves not only "resisting damage" but also the capacity to "actively dissipate" excess light energy to protect the photosynthetic apparatus, and ChlF technology is uniquely positioned to capture information from both dimensions.

Expanding the view from homogeneous controlled laboratory environments to complex natural or agricultural systems, ChlF technology unveils the layered complexity of plant responses to waterlogging. The study by Mao *et al.* (2023) on *Spartina alterniflora* in the intertidal zone is a prime example. Fully submerged bottom leaves suffered a drastic plunge of over 30% in $Y_{(II)}$, with only 20% of PSII reaction centers remaining active, whereas performance decline in top leaves exposed to air was minimal. This stark contrast strongly cautions that the intense spatial heterogeneity within a plant or canopy must be considered when assessing overall waterlogging stress. Fluorescence imaging technology demonstrates unique advantages here, as confirmed by Wu *et al.* (2024), showing differential sensitivity of $Y_{(II)}$ to waterlogging across segments of peanut leaves.

The plant's physiological clock or growth stage profoundly modulates the fluorescence response. Winter wheat experiencing waterlogging at the tillering stage showed severe impairment in both fluorescence parameters and yield, whereas mild waterlogging at the grain-filling stage could even enhance photosynthetic capacity (Wu *et al.* 2015). This seemingly paradoxical result astutely illustrates that the ultimate impact of waterlogging stress is co-determined by the interaction between stress intensity and the plant's intrinsic physiological demands; thus, discussing "waterlogging damage" in isolation from the growth stage may lead to biased conclusions.

New research has attempted to integrate ChlF parameters with more profound bioenergetic metrics to

Table 5. Applications of ChlF in evaluating mitigation strategies for salinity stress.

Plant	Processing conditions	Main responses and changes	Reference
Rapeseed	Foliar spraying of glycine betaine	Glycine betaine application significantly enhances fluorescence parameters and photosynthetic efficiency, and provides rapid fluorescent assessment indicators for the physiological effects of glycine betaine.	Athar *et al.* 2015
Mung bean	Foliar spraying of salicylic acid + silicon	Salicylic acid and silicon synergistically regulated ion balance and enhanced complex activity more effectively than silicon alone while suppressing PSII photodamage, as evidenced by elevated F_0 and reduced PI, and ultimately achieved joint protection of PSII through SA–Si coordination.	Ghassemi-Golezani and Lotfi 2015

Table 6. Summary of ChlF applications in sensing water stress.

Plant	Main responses and changes	Reference
Peanut	Prolonged waterlogging leads to a significant decline in F_V/F_m and ETR, indicating damage to PSII reaction centers and inhibition of linear electron transport. Short-term waterlogging (3–5 d) may slightly promote photosynthesis, but sustained stress (7–9 d) causes inhibitory effects. The sensitivity of $Y_{(II)}$ to waterlogging varies across different segments of the leaves.	Zhang *et al.* 2019, Sharma *et al.* 2022, Wu *et al.* 2024
Sorghum	Prolonged waterlogging leads to a significant decline in F_V/F_m and ETR, indicating damage to PSII reaction centers and inhibition of linear electron transport.	Zhang *et al.* 2019, Sharma *et al.* 2022
Pumpkin	The waterlogging-tolerant cultivar 'EP' showed significantly smaller declines in F_V/F_m and $Y_{(II)}$ compared to the sensitive cultivar. NDVI and PRI were highly correlated with core ChlF parameters.	Lin *et al.* 2020
North China landscape shrubs	Tolerant species not only maintained stable QY but also exhibited an active increase in NPQ during later stress stages to dissipate excess light energy.	Fang *et al.* 2022
Winter wheat	Waterlogging at the tillering stage showed severe impairment in both ChlF parameters and yield, whereas mild waterlogging at the grain-filling stage could even enhance photosynthetic capacity, indicating the growth stage profoundly modulates the ChlF response.	Wu *et al.* 2015
Mulberry seedlings	The decline in F_V/F_m and the rise in NPQ were accompanied by fine-tuning of the thylakoid proton motive force components (*e.g.*, decreased thylakoid proton conductivity and increased total electrochromic shift), revealing an actively initiated energy regulation program.	Li and Rao 2024

Table 7. Summary of ChlF applications in sensing toxicity stress.

Plant	Stress conditions	Main responses and changes	Reference
Wheat	Cd, Zn	Cadmium inhibits the energy conversion efficiency of PSII, reducing it by 4–5 times; zinc disrupts the integrity of the electron transport chain.	Paunov *et al.* 2018
Duckweed	Cu	Low concentrations (5–10 μmol L^{-1}) increased $Y_{(II)}$ and ETR; high concentration (100 μmol L^{-1}) damaged the PSII reaction center, and F_V/F_m was significantly changed.	Singh *et al.* 2022
Chlorella pyrenoidosa	Triazine herbicides	A novel photosynthetic response index based on the OJIP curve showed significantly higher sensitivity than traditional parameters (F_V/F_m, PI_{abs}), and could reliably distinguish different toxicity levels.	Gan *et al.* 2023
Chlamydomonas reinhardtii	Diuron (DCMU)	Very low concentrations not only inhibited PSII electron transport but might also disrupt normal physiological regulation, suggesting the need to re-evaluate its environmental safety concentration.	Grasso *et al.* 2022
Sugar beet seedlings	Fomesafen	Dose-dependent inhibition of F_V/F_m and $Y_{(II)}$; concentrations ≥0.025 mg kg^{-1} significantly affect photosynthetic performance.	Li *et al.* 2022

delineate a complete picture of plant stress resistance. The study by Li and Rao (2024) on mulberry seedlings represents this direction. They found that the decline in F_V/F_m and the rise in NPQ under waterlogging stress were accompanied by fine-tuning of the components of the thylakoid proton motive force (pmf) (*e.g.*, decreased thylakoid proton conductivity and increased total electrochromic shift). This linkage elucidates that plants do not passively endure PSII functional damage; instead, they actively initiate a comprehensive energy regulation process from reaction centers to the trans-thylakoid proton gradient to balance the conflict between light capture and carbon assimilation demands.

Toxicity stress

Toxicity stresses, such as metals, nanoparticles, and organic pollutants, are critical environmental factors that limit crop growth and productivity. These stresses induce significant disruptions in photosynthesis through multiple mechanisms, including damage to thylakoid membrane structure, inhibition of PSII reaction center activity, and interference with Rubisco enzyme function (Muhammad *et al.* 2021, Pathak *et al.* 2022). A summary of ChlF applications in sensing toxicity stress is given in Table 7.

Studies have shown that metal toxicity significantly affects the plastoquinone pool size and the efficiency of electron transfer to PSI, with specific ChlF parameters serving as sensitive indicators of such stresses (Faseela *et al.* 2020, Dąbrowski *et al.* 2023, Kalisz *et al.* 2023). Importantly, different metal elements exhibit distinct "fingerprints" in their interference with the photosynthetic electron transport chain. For instance, cadmium (Cd) stress primarily and strongly suppresses fluorescence parameters related to the energy conversion efficiency of PSII, whereas zinc (Zn) tends to more significantly impair the integrity of the electron transport chain. Such differences can be clearly distinguished through meticulous fluorescence analysis (Bayçu *et al.* 2017, Paunov *et al.* 2018). This specificity suggests that ChlF technology acts not merely as an alarm for "stress presence" but also as a diagnostic tool for identifying "stress type".

Dynamic changes in ChlF parameters show the concentration-dependent effects of metal stress. In duckweed's response to copper (Cu), where $Y_{(II)}$ and ETR can be stimulated at low concentrations, while high concentrations cause irreversible damage to reaction centers (Singh *et al.* 2022). This biphasic concentration response underscores the critical importance of strictly defining the effective diagnostic range when using fluorescence parameters to assess metal toxicity, thereby avoiding misinterpretation of adaptive responses at low concentrations.

In the case of organic pollutant stress, ChlF technology also demonstrates high sensitivity (Lukatkin *et al.* 2023, Mikulka *et al.* 2024). For example, a novel photosynthetic response index, constructed based on the OJIP curve of *Chlorella pyrenoidosa*, shows significantly higher sensitivity for detecting triazine herbicides (such as atrazine) compared to traditional parameters like F_V/F_m and PI_{abs}, and can reliably distinguish different toxicity levels, offering a new technical approach for water pollution monitoring (Gan *et al.* 2023). Another detailed study using steady-state and transient fluorescence kinetics in *Chlamydomonas reinhardtii* found that very low concentrations of diuron (DCMU) not only inhibit PSII electron transport but may also disrupt normal physiological regulation in plants, suggesting that its environmental safety concentration needs re-evaluation (Grasso *et al.* 2022).

Pot experiments further confirmed that fomesafen residues inhibit key parameters such as F_V/F_m and $Y_{(II)}$ in sugar beet seedlings in a dose-dependent manner, with concentrations ≥ 0.025 mg kg^{-1} significantly affecting photosynthetic performance (Li *et al.* 2022). Additionally, the JIP-test parameter system can successfully differentiate the specific toxicity targets of different herbicides on the donor side and reaction center of PSII (Hassannejad *et al.* 2020). These findings demonstrate that ChlF technology is not only a tool for determining "whether plants are injured" but also a key means of elucidating "how pollutants exert toxic effects".

Importantly, this technology is also widely used to study the alleviation mechanisms of toxicity stress by exogenous substances and plants themselves. For example, Fe_2O_3 nanoparticles were shown to enhance the photosynthetic efficiency of oak trees by increasing PI_{abs} and the electron flux per reaction center (ET_0/RC) (Kalisz *et al.* 2023). Similarly, the mitigating effect of exogenous nitric oxide (NO) on hexavalent chromium (Cr^{6+}) toxicity was reflected in its significant repair of damage on both the donor and acceptor sides of PSII, along with improvements in a series of related fluorescence parameters (Huang *et al.* 2018). These findings establish the practical value of ChlF in quantifying the efficacy of stress-mitigating agents and screening efficient alleviation strategies.

Mitigation strategies developed by plants themselves can also be interpreted through fluorescence parameters. The acclimation mechanism in lettuce under Cd stress, involving enhanced thermal dissipation to alleviate PSII damage, was directly reflected in adjustments of NPQ-related fluorescence parameters (Zhou *et al.* 2024). This reveals that when facing metal stress, the photosynthetic machinery is not entirely passive; rather, it activates a series of defensive mechanisms involving active energy dissipation and redistribution.

Nitrogen stress

Damage to the photosynthetic apparatus under nitrogen (N) stress can be sensitively detected from ChlF. By quantifying changes in key fluorescence parameters such as F_V/F_m and $Y_{(II)}$, ChlF provides critical insights for optimizing agricultural practices and enabling precise nitrogen fertilizer management (Noga *et al.* 2017, Chen *et al.* 2022, Zhang *et al.* 2022, Hu *et al.* 2025). A summary of ChlF applications in evaluating nitrogen stress is given in Table 8.

Extensive research has confirmed that nitrogen deficiency directly impairs the core function of PSII

Table 8. Summary of ChlF applications in sensing nitrogen stress.

Plant	Main responses and changes	Reference
Tea plant	N deficiency led to a decline at the P-step of the ChlF transient curve, significant reductions in F_V/F_m, ET_0/ABS, and PI_{abs}, along with increases in DI_0/RC, indicating blockages in the photosynthetic electron transport chain and decreased reaction center activity.	Lin *et al.* 2016
Peanut	Low-N stress significantly reduced F_V/F_m and PI_{abs}; the process involved specific regulatory modules composed of miRNAs and their target genes.	Kong *et al.* 2025
Rice (low-N-tolerant cultivar)	Low-N tolerant genotypes (*e.g.*, CR Dhan 311) optimized the regulation of both q_P and NPQ, maintaining relatively higher photosynthetic efficiency under low N conditions. The tolerant cultivar Jijing 88 demonstrated smaller fluctuations and higher stability in parameters such as $Y_{(II)}$, F_V'/F_m', and ETR during recovery from stress.	Tantray *et al.* 2020, Qi *et al.* 2025
Soybean	Under N stress, ChlF parameters (*e.g.*, F_V/F_m, $Y_{(II)}$, q_P) showed only slight suppression, and the changes were not significant, having a potential strategy of reallocating resources to prioritize reproductive growth.	Bu *et al.* 2023
Sorghum	Application of exogenous BR significantly improved ChlF characteristics under low-N conditions, evidenced by decreased F_0, F_m, and NPQ, alongside an increase in F_V/F_m, and a synergistic enhancement of electron transport efficiency between PSI and PSII.	Hu *et al.* 2025

(Markou *et al.* 2017). For instance, in N-deficient tea plants, the ChlF transient curve often exhibits a decline at the P-step, accompanied by significant reductions in F_V/F_m, ET_0/ABS, and PI_{abs}, along with increases in energy dissipation parameters (DI_0/RC). These changes consistently indicate blockages in the photosynthetic electron transport chain and decreased reaction center activity, representing key internal mechanisms through which N stress inhibits CO_2 assimilation capacity (Lin *et al.* 2016). In a study on peanut seedlings, Kong *et al.* (2025) found that low-N stress not only significantly reduced F_V/F_m and PI_{abs} but also involved specific regulatory modules composed of miRNAs and their target genes, offering a new molecular perspective on the ChlF response to N stress.

Under N stress, different plant species and even genotypes exhibit diverse fluorescence responses and adaptive strategies. A comparative study between low-N tolerant rice cultivar CR Dhan 311 and sensitive cultivar Rasi revealed that the tolerant genotype optimizes the regulation of both q_P and NPQ, thereby maintaining relatively higher photosynthetic efficiency under low-N conditions, highlighting the plasticity of the photosynthetic apparatus (Tantray *et al.* 2020). This genotypic variation is also evident during recovery from N stress. The low-N tolerant rice cultivar Jijing 88 demonstrated smaller fluctuations and greater stability in parameters such as $Y_{(II)}$, the efficiency of excitation energy capture by open PSII reaction centers (F_V'/F_m') and ETR (Qi *et al.* 2025).

Notably, the photosynthetic apparatus can sometimes display considerable resilience. For instance, when soybeans are exposed to nitrogen stress, ChlF parameters (*e.g.*, F_V/F_m, $Y_{(II)}$, q_P) exhibit slight inhibition but the changes are not significant. Instead, soybeans adjust their energy allocation strategy, prioritizing resource allocation to reproductive growth, thereby ensuring population persistence (Bu *et al.* 2023). This finding indicates that "status" parameters like F_V/F_m alone may be insufficient to reveal the full spectrum of a plant's survival strategies under stress, while "flux" parameters (such as PI_{abs}) reflecting energy flow and photoprotective mechanisms (NPQ) may provide a more comprehensive picture.

In complex field environments, N stress often co-occurs with other abiotic stresses, forming combined stress scenarios. Research on lichen *Cladonia rei* demonstrated that environmental nitrogen concentrations significantly modulate the organism's response to and recovery from salt stress. The decline in F_V/F_m caused by short-term salt stress under low N was reversible, whereas high N hindered this recovery process and exacerbated fluctuations in fluorescence parameters in habitats with heavy metal contamination (Chowaniec *et al.* 2023). This finding underscores the critical and complex role of nitrogen management in ecosystems experiencing combined stresses.

To more accurately diagnose combined stresses, integrating multi-source information has emerged as a trend (Masseroni *et al.* 2017). For instance, in a study on combined N and salt stress in forage rape, combining ChlF parameters with hyperspectral data to construct a random forest model significantly improved the estimation accuracy of key photosynthetic traits, including PSII photochemical efficiency and electron transport rate (Wang *et al.* 2025b).

Notably, the application of exogenous substances has proven to be an effective strategy for alleviating N stress in crops. For example, brassinolide (BR) application significantly improved ChlF characteristics in sorghum seedlings under low-N conditions. This was evidenced by decreased F_0, F_m, and NPQ, alongside an increase in F_V/F_m, and a synergistic enhancement of electron transport efficiency between PSI and PSII (Hu *et al.* 2025). The response of these fluorescence parameters clearly reveals that BR's effect extends beyond the repair of a single photosystem, systematically enhancing the overall operational efficiency and stability of the photosynthetic apparatus under N-deficient conditions. This finding not only provides direct evidence supporting the use of BR as a growth regulator in stress-resistant production but also suggests that manipulating the energy flow within the photosynthetic apparatus *via* exogenous substances could be a viable approach to compensate for insufficient nitrogen nutrition and maintain crop photosynthetic productivity.

Discussion and future research opportunities

This review analyzes ChlF research over the past decade, systematically summarizing the relationships among ChlF parameters, stress types, and their corresponding physiological responses. Compared to the previous decade, from 2005 to 2015, the achievements are substantial. (*1*) With the continuous development and widespread applications of ChlF-based technologies, ChlF has been more frequently used to study combined stresses in plants. (*2*) Compared to earlier studies that focused predominantly on phenomenological observations, recent work has increasingly concentrated on using changes in ChlF parameters to interpret plant physiological mechanisms. (*3*) ChlF is not only used to sense plant responses to stresses, but also used as a feedback indicator to design strategies to alleviate stresses.

In recent plant stress biology research, the intrinsic mechanisms underlying combined stress remain insufficiently understood, representing a significant fundamental bottleneck in the field. For instance, Zhou et al. (2018b) demonstrated that the synergistic damaging effect of combined stress on plant reproductive organs is significantly higher than that of individual stresses. However, under conditions where multiple stresses coexist, methods to effectively dissect the contribution of each individual stressor are still lacking. Although ChlF technology has yielded substantial results in plant physiological response studies, ChlF measurements are often limited to measurements in the laboratory. These dual constraints – fundamental understanding and technological capability – significantly hinder the effective application of this technology in complex agricultural environments. Therefore, establishing a ChlF research framework capable of systematically analyzing the influence of complex environmental factors, and developing fluorescence diagnostic technologies that combine high-throughput capabilities with high resolution at field scales have become critical frontiers in advancing this field from theoretical breakthroughs to practical application.

Over the past decade, the rapid advancement of machine learning (ML) technologies has significantly propelled the frontiers of plant physiology research, demonstrating considerable potential particularly in the field of chlorophyll fluorescence analysis (Cen *et al.* 2017, 2022; Gill *et al.* 2022, Wang *et al.* 2023). Dąbrowski *et al.* (2024) suggested that nonlinear algorithms, such as Random Forest, Support Vector Machines, and Artificial Neural Networks, significantly outperform traditional linear methods in processing chlorophyll fluorescence data for predicting complex traits like yield loss. Concurrently, the integration of machine learning with chlorophyll fluorescence techniques enables the direct extraction of robust features from high-dimensional, noisy OJIP transient curves captured by high-throughput phenotyping platforms (Tran 2024). This substantially reduces reliance on tedious data pre-processing and manual selection of points, thereby providing unprecedented sensitivity and novel pathways for early stress diagnosis and the differentiation of combined stresses (Xia *et al.* 2023, Keller *et al.* 2025). Lotfi *et al.* (2024) successfully elucidated the mechanisms by which environmental factors influence key fluorescence parameters, NPQ and $Y_{(II)}$, using Classification and Regression Tree analysis. Furthermore, deep learning-based image processing techniques have extended chlorophyll fluorescence analysis from mere temporal kinetics to high-resolution spatial distribution, enabling the precise visualization and quantification of photosynthetic heterogeneity, thus providing an intuitive basis for understanding stress response mechanisms at the leaf level (McAusland *et al.* 2019, Moustakas *et al.* 2021).

However, for this interdisciplinary field to mature and achieve widespread application, several key challenges must be overcome. The primary obstacle is the data bottleneck – the acquisition of high-quality, large-scale chlorophyll fluorescence imaging data with precise physiological annotations is costly, and controlling variables is complex (Jiang *et al.* 2024). Secondly, machine learning models, especially deep learning, are often perceived as "black boxes"; the disconnect between their decision logic and underlying biological mechanisms limits their value as tools for scientific discovery (Esser-Skala and Fortelny 2023). Additionally, models are prone to overfitting with limited data, and their generalization capability across species and environmental conditions needs further validation through systematic benchmarking (Mostafa *et al.* 2023, Khatibi and Ali 2024).

Conclusion

Advances in the applications of ChlF from PSII over the past decade were summarized. ChlF continues to serve as an indispensable tool across multiple areas of plant research as a result of its nondestructive nature and sensitive probing capabilities for photosynthetic processes. However, current mainstream fluorescence parameters remain limited in their ability to interpret complex physiological mechanisms in complex conditions. Emerging machine learning approaches are called for to overcome the limitations, enabling visualization and real-time monitoring of cryptic physiological processes in plants, environment evaluation, and agriculture through advanced feature extraction and data processing.

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