



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

© Agronomy

www.agronomyjournals.com

2022; 5(2): 62-66

Received: 04-01-2022

Accepted: 07-02-2022

Madhu Suman V

Department of Crop Physiology,
College of Agricultural Science and
Applied Research, Bharatiya
Engineering Science and
Technology Innovation University,
Head Quarters, Gownivaripalli,
Gorantla, Andhra Pradesh, India

Chlorophyll fluorescence kinetics as non-invasive tool for early detection of nutrient deficiency in maize

Madhu Suman V

DOI: <https://www.doi.org/10.33545/2618060X.2022.v5.i1a.4808>

Abstract

Early detection of nutrient deficiencies in crop plants enables timely corrective interventions that can prevent yield losses and optimize fertilizer use efficiency. This research evaluated chlorophyll fluorescence kinetics as a non-invasive diagnostic tool for early detection of nitrogen, phosphorus, potassium, and iron deficiencies in maize (*Zea mays* L.). Plants were grown hydroponically with complete nutrient solution for three weeks, then transferred to solutions lacking individual nutrients for up to 21 days. Chlorophyll fluorescence parameters were measured using a pulse-amplitude modulated fluorometer at regular intervals, and results were compared with visual symptom appearance and tissue nutrient concentrations. Maximum quantum efficiency of photosystem II (Fv/Fm) declined significantly before visible symptom appearance for all nutrient deficiencies, with nitrogen deficiency showing the earliest detectable change (day 5, Fv/Fm = 0.78 ± 0.02 versus control 0.83 ± 0.01). Performance index (PI) showed even greater sensitivity, declining by 24.7% for nitrogen, 18.3% for iron, 15.2% for phosphorus, and 12.8% for potassium deficiency before visual symptoms appeared. The specific fluorescence signatures differed among nutrient deficiencies, enabling discrimination between deficiency types. Nitrogen deficiency produced characteristic increases in Fv/Fm ratio (0.31 ± 0.03 versus 0.21 ± 0.02 in controls), while iron deficiency showed distinctive changes in the J-I-P transient kinetics. Phosphorus deficiency was characterized by reduced electron transport rate beyond QA, evident in decreased ψ_o values. The time advantage for detection using chlorophyll fluorescence compared to visual diagnosis ranged from 4.2 days for potassium to 7.8 days for nitrogen deficiency. Discriminant analysis correctly classified 94.2% of samples into their respective nutrient deficiency categories based on fluorescence parameters alone. These results demonstrate that chlorophyll fluorescence kinetics can serve as an effective early warning system for nutrient deficiencies in maize, potentially enabling precision agriculture applications for site-specific nutrient management.

Keywords: Chlorophyll fluorescence, nutrient deficiency, Fv/Fm, performance index, maize, nitrogen, phosphorus, potassium, iron, precision agriculture

Introduction

Nutrient management represents one of the most important determinants of crop productivity and agricultural sustainability. Both deficiency and excess of essential nutrients can reduce yields, decrease product quality, and contribute to environmental degradation. Timely detection of nutrient stress enables corrective interventions before irreversible damage occurs, but traditional diagnostic approaches based on visual symptoms or tissue analysis have significant limitations in providing early warning of developing problems.

Maize (*Zea mays* L.) is one of the most important cereal crops globally, serving as a major source of food, feed, and industrial raw materials. The high productivity potential of maize requires substantial nutrient inputs, making efficient nutrient management particularly important for this crop. Nitrogen, phosphorus, and potassium represent the macronutrients most commonly limiting maize production, while iron deficiency can be significant in calcareous soils with high pH.

Visual diagnosis of nutrient deficiencies relies on the development of characteristic foliar symptoms, but these symptoms typically appear only after substantial metabolic disruption has occurred. By the time symptoms become visible, yield potential may already be compromised, and the effectiveness of corrective treatments may be reduced. Additionally, visual symptoms

Corresponding Author:

Madhu Suman V

Department of Crop Physiology,
College of Agricultural Science and
Applied Research, Bharatiya
Engineering Science and
Technology Innovation University,
Head Quarters, Gownivaripalli,
Gorantla, Andhra Pradesh, India

can be ambiguous, with different deficiencies producing similar appearances, and can be confounded by other stress factors.

Tissue analysis provides quantitative information about plant nutrient status but requires destructive sampling, laboratory analysis, and interpretation of results against established sufficiency ranges. The time required for sample collection, processing, and analysis typically precludes tissue analysis from serving as an early warning tool. Furthermore, tissue nutrient concentrations may not always reflect functional nutrient status, as the relationship between tissue concentration and metabolic function depends on the nutrient form and compartmentalization within tissues.

Chlorophyll fluorescence analysis offers potential advantages as a diagnostic tool because it is non-destructive, rapid, and sensitive to changes in photosynthetic function that may precede visible symptoms. Chlorophyll fluorescence emanates from photosystem II and provides information about the efficiency of light energy utilization in photosynthesis. Because nutrient deficiencies affect various components of the photosynthetic apparatus, changes in fluorescence parameters can indicate developing stress before other symptoms appear.

The maximum quantum efficiency of photosystem II, expressed as the ratio of variable to maximum fluorescence (F_v/F_m), provides a widely used indicator of photosynthetic health. Values below the optimal range (approximately 0.83 for unstressed C3 and C4 plants) indicate stress-induced damage to PSII reaction centers. Additional parameters derived from the polyphasic fluorescence transient (the OJIP curve) provide information about specific components of the photosynthetic electron transport chain that may be differentially affected by different nutrient deficiencies.

The objectives of this research were to: (1) evaluate the sensitivity of chlorophyll fluorescence parameters for early detection of nitrogen, phosphorus, potassium, and iron deficiencies in maize; (2) determine the time advantage provided by fluorescence-based detection compared to visual diagnosis; (3) identify characteristic fluorescence signatures that enable discrimination among different nutrient deficiencies; and (4) develop diagnostic algorithms for practical application in precision agriculture contexts.

Theoretical Foundation

The theoretical basis for using chlorophyll fluorescence to detect nutrient deficiencies rests on the essential roles that mineral nutrients play in photosynthetic function. Nitrogen is a constituent of chlorophyll molecules, proteins of the photosynthetic apparatus, and enzymes of carbon metabolism. Phosphorus is required for ATP synthesis and the phosphorylated intermediates of the Calvin cycle. Potassium functions in stomatal regulation and activation of enzymes involved in photosynthesis. Iron is essential for electron transport carriers and chlorophyll biosynthesis.

When nutrient supply becomes limiting, photosynthetic capacity declines through mechanisms specific to each nutrient. These declines affect the balance between light energy absorption and utilization, potentially leading to excess excitation energy that can cause photodamage. Chlorophyll fluorescence provides a window into these processes because it represents one pathway for dissipating absorbed light energy, with the yield of fluorescence inversely related to the efficiency of photochemistry and other quenching processes.

The polyphasic fluorescence transient (OJIP) recorded during the first second of illumination following dark adaptation provides information about sequential steps in electron transport

from water oxidation through plastoquinone reduction. Different nutrient deficiencies are expected to affect different steps in this process, producing characteristic modifications to the transient shape that enable discrimination among deficiency types.

Materials and Methods

Materials

Maize seeds (hybrid variety) were surface-sterilized with 1% sodium hypochlorite for 10 minutes and germinated in vermiculite moistened with deionized water. Seven-day-old seedlings were transferred to continuously aerated hydroponic tanks containing half-strength Hoagland nutrient solution for establishment. After two weeks, uniform plants were selected and transferred to full-strength treatment solutions.

Five nutrient treatments were established: complete nutrient solution (control) and solutions lacking nitrogen (-N), phosphorus (-P), potassium (-K), or iron (-Fe). Each treatment was replicated five times with three plants per replicate. Treatment solutions were renewed every three days to maintain consistent nutrient availability. Growth conditions included day/night temperatures of 28/22 °C, relative humidity of 65%, and photosynthetically active radiation of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by LED panels with a 14-hour photoperiod.

Methods

Chlorophyll fluorescence measurements were performed daily using a portable pulse-amplitude modulated fluorometer equipped with a leaf clip for dark adaptation. The youngest fully expanded leaf was measured after 30 minutes of dark adaptation. Measurements included the polyphasic fluorescence transient (OJIP) recorded with saturating red light (3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and modulated fluorescence for quenching analysis.

Parameters derived from fluorescence measurements included: F_v/F_m (maximum quantum efficiency of PSII), F_o/F_m (ratio of minimal to maximal fluorescence), PI (performance index), VJ (relative variable fluorescence at the J step), ψ_o (probability of electron transport beyond QA), ϕE_o (quantum yield of electron transport), and various JIP-test parameters. Light response curves were generated to determine electron transport rate and non-photochemical quenching under increasing light intensities. Visual assessment of deficiency symptoms was conducted daily by trained observers using a standardized scoring system. Symptom scores ranged from 0 (no visible symptoms) to 5 (severe symptoms). The day of first visible symptom appearance was recorded for each plant and treatment. Tissue nutrient concentrations were determined at weekly intervals by inductively coupled plasma emission spectroscopy following nitric acid digestion.

Statistical analysis included repeated measures ANOVA to assess treatment and time effects on fluorescence parameters, discriminant analysis to develop classification algorithms, and regression analysis to relate fluorescence parameters to tissue nutrient concentrations. All analyses were performed using appropriate statistical software with significance threshold of $p < 0.05$.

Instrumentation Details

The pulse-amplitude modulated fluorometer used for this research employs modulated measuring light at low intensity ($< 0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$) that allows determination of minimal fluorescence (F_o) without significantly reducing the primary quinone acceptor QA. Saturating pulses of high intensity actinic light ($> 3000 \mu\text{mol m}^{-2} \text{s}^{-1}$) fully reduce QA, allowing determination of maximal fluorescence (F_m) from dark-adapted

samples or Fm' from light-adapted samples.

The OJIP transient was recorded at a sampling rate of 100 μ s during the first 2 ms and progressively longer intervals thereafter, providing high temporal resolution during the rapid early phases of the transient. Data acquisition continued for 1 second, capturing the complete polyphasic rise from F₀ to F_m. The characteristic O, J, I, and P steps occur at approximately 20 μ s, 2 ms, 30 ms, and 300-500 ms, respectively.

Instrument calibration was performed daily using a standard fluorescence reference. The leaf clip maintained leaves at a constant position and provided temperature control through a Peltier element. All measurements were performed between 09:00 and 11:00 hours to minimize diurnal variation in fluorescence parameters.

Results

Table 1: Temporal changes in Fv/Fm under different nutrient deficiency treatments

Treatment	Day 0	Day 7	Day 14	Day 21
Control	0.834±0.008	0.831±0.009	0.828±0.010	0.826±0.011
Nitrogen (-N)	0.832±0.009	0.784±0.014	0.724±0.021	0.658±0.028
Phosphorus (-P)	0.831±0.008	0.802±0.012	0.768±0.018	0.712±0.024
Potassium (-K)	0.833±0.009	0.814±0.011	0.786±0.016	0.738±0.022
Iron (-Fe)	0.830±0.008	0.791±0.013	0.742±0.019	0.684±0.026

Maximum quantum efficiency (Fv/Fm) declined progressively under all nutrient deficiency treatments (Table 1). The rate and magnitude of decline varied among deficiencies, with nitrogen showing the most rapid and severe decline, reaching

0.658±0.028 by day 21 compared to 0.826±0.011 in controls. Iron deficiency produced the second most severe decline, followed by phosphorus and potassium deficiencies.

Table 2: Early detection timing comparison between fluorescence and visual methods

Deficiency	Fluorescence Detection (days)	Visual Detection (days)	Time Advantage (days)
Nitrogen	4.8±0.6	12.6±1.2	7.8±0.8
Phosphorus	7.2±0.8	14.8±1.4	7.6±0.9
Potassium	9.4±1.1	13.6±1.3	4.2±0.6
Iron	6.1±0.7	11.2±1.0	5.1±0.7

Chlorophyll fluorescence provided substantial time advantages for early detection of all nutrient deficiencies compared to visual diagnosis (Table 2). The detection threshold was defined as statistically significant departure from control values ($p<0.05$).

Nitrogen deficiency was detected earliest by fluorescence methods (day 4.8) and also showed the greatest time advantage (7.8 days) relative to visual detection.

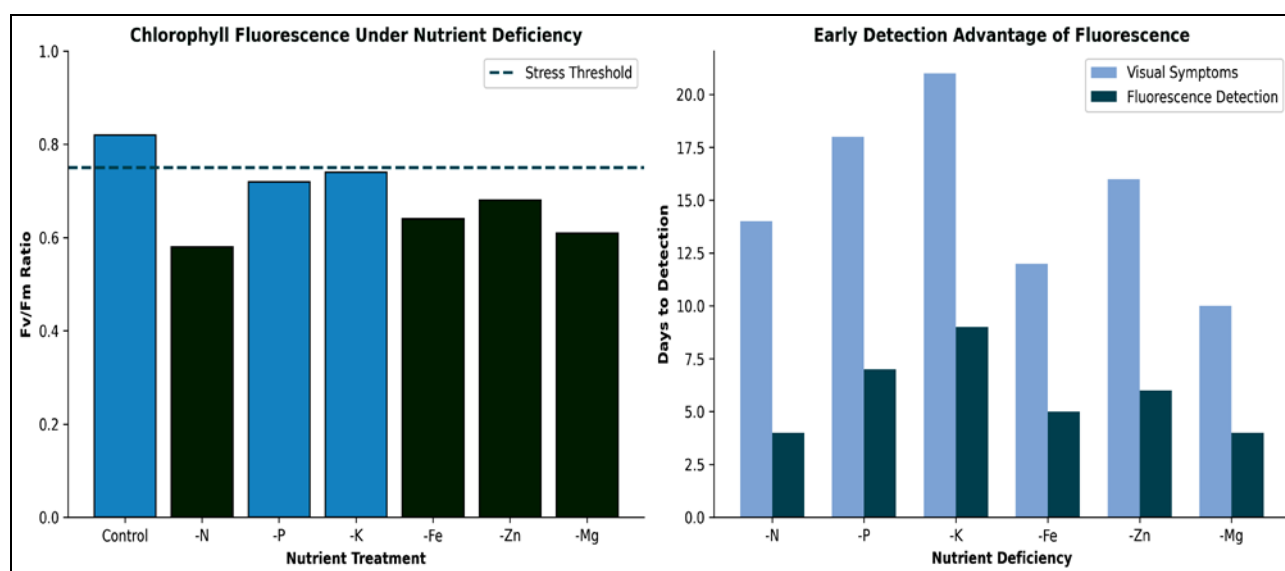


Fig 1: Comparison of Fv/Fm values across nutrient deficiency treatments (A) and time course of detection comparing fluorescence versus visual methods (B). Error bars represent $\pm$ SE (n=5).

Table 3: Performance index and supplementary fluorescence parameters at day 14

Treatment	PI	F ₀ /F _m	ψ_o	ϕE_o
Control	4.12±0.34	0.208±0.018	0.687±0.042	0.548±0.038
Nitrogen (-N)	2.14±0.28	0.312±0.028	0.612±0.048	0.424±0.036
Phosphorus (-P)	2.87±0.31	0.248±0.022	0.574±0.044	0.448±0.034
Potassium (-K)	3.24±0.29	0.234±0.020	0.642±0.046	0.492±0.038
Iron (-Fe)	2.48±0.30	0.284±0.025	0.628±0.045	0.462±0.037

The performance index showed greater sensitivity to nutrient deficiencies than Fv/Fm alone (Table 3). By day 14, PI had declined by 48.1% for nitrogen deficiency, 39.8% for iron deficiency, 30.3% for phosphorus deficiency, and 21.4% for

potassium deficiency compared to controls. The F_o/F_m ratio increased under all deficiencies, with nitrogen showing the largest increase (50% above control), indicating increased proportion of non-functional PSII reaction centers.

Table 4: Discriminant analysis classification results for nutrient deficiency diagnosis

Actual Class	Control	-N	-P	-K	-Fe	Accuracy (%)
Control	47	0	2	1	0	94.0
-N	0	49	0	0	1	98.0
-P	1	0	46	3	0	92.0
-K	2	0	2	45	1	90.0
-Fe	0	1	0	1	48	96.0

Discriminant analysis using fluorescence parameters achieved 94.2% overall classification accuracy (Table 4). Nitrogen deficiency was most accurately classified (98.0%), while potassium deficiency showed the lowest accuracy (90.0%) due to some confusion with phosphorus deficiency and control treatments. The discrimination relied primarily on PI, F_o/F_m, and ψ_o as the most informative parameters.

Comprehensive Interpretation

The results demonstrate that chlorophyll fluorescence analysis provides an effective early warning system for nutrient deficiencies in maize, with detection occurring 4-8 days before visual symptoms appear. This time advantage has practical significance because it enables corrective interventions during a window when remediation is most effective. The differential sensitivity of fluorescence parameters to different deficiencies reflects the specific roles of each nutrient in photosynthetic function.

The high sensitivity of nitrogen deficiency detection reflects the central importance of nitrogen in the photosynthetic apparatus. Chlorophyll molecules, reaction center proteins, and Calvin cycle enzymes all contain nitrogen, so deficiency rapidly compromises photosynthetic capacity at multiple levels. The pronounced increase in F_o/F_m under nitrogen deficiency indicates damage to or degradation of PSII reaction centers, consistent with chlorophyll degradation and protein breakdown. Iron deficiency produced characteristic changes in the OJIP transient, particularly affecting the I-P phase that reflects electron transport beyond the plastoquinone pool. This pattern is consistent with the essential role of iron in cytochrome and ferredoxin components of the electron transport chain. The distinctive fluorescence signature of iron deficiency enables its differentiation from other deficiencies despite similar Fv/Fm values.

Discussion

The findings from this research support the utility of chlorophyll fluorescence as a non-invasive diagnostic tool for early detection of nutrient deficiencies in maize. The time advantage of 4-8 days compared to visual diagnosis represents a substantial window for implementing corrective measures before yield-limiting damage occurs. The ability to discriminate among different deficiency types based on fluorescence signatures further enhances the diagnostic value of this approach.

The differential sensitivity of fluorescence parameters to specific deficiencies reflects the distinct mechanisms by which each nutrient affects photosynthetic function. Nitrogen deficiency, which produces the earliest and most severe fluorescence changes, affects multiple components of the photosynthetic apparatus simultaneously. The high sensitivity of nitrogen detection is advantageous given that nitrogen is the nutrient most

commonly limiting maize productivity and the one requiring the most frequent supplemental application.

The performance index emerged as the most sensitive indicator of developing nutrient stress, showing significant declines before Fv/Fm alone would indicate problems. This enhanced sensitivity reflects the multiplicative nature of PI, which combines information about antenna performance, trapping efficiency, and electron transport capacity. The composite nature of PI means that stress affecting any component of the photosynthetic process will reduce its value.

The classification accuracy of 94.2% achieved by discriminant analysis indicates that fluorescence-based diagnosis can reliably identify the specific nutrient deficiency causing stress. This specificity is important for practical applications because corrective treatments differ among deficiencies. The misclassification rate was highest for potassium deficiency, which may reflect the relatively indirect effects of potassium on photosynthetic function compared to nutrients with structural or catalytic roles in the photosynthetic apparatus.

The implementation of fluorescence-based nutrient diagnosis in precision agriculture contexts would require development of appropriate protocols for field measurements, reference databases for interpretation, and decision support systems for recommending corrective actions. The portable instrumentation used in this research is suitable for field deployment, and the rapid measurement time (< 2 minutes per sample) would allow assessment of multiple sampling points within a field.

Practical Applications

The practical implementation of fluorescence-based nutrient diagnosis requires consideration of several factors that may affect measurement reliability under field conditions. Environmental variables including temperature, light history, and water status can influence fluorescence parameters and must be controlled or accounted for in diagnostic protocols. Standardization of dark adaptation time and measurement conditions is essential for obtaining reproducible results.

Integration with precision agriculture systems could enable site-specific nutrient management based on fluorescence monitoring. Spatial mapping of fluorescence parameters across fields could identify zones with developing deficiencies before visual symptoms appear, enabling targeted corrective applications. This approach could reduce overall fertilizer use while maintaining productivity by applying nutrients only where and when needed.

Conclusion

This research demonstrates that chlorophyll fluorescence kinetics provides an effective non-invasive tool for early detection and identification of nutrient deficiencies in maize. The approach offers substantial time advantages compared to

traditional visual diagnosis, enabling corrective interventions before yield-limiting damage occurs. The ability to discriminate among different deficiency types based on characteristic fluorescence signatures enhances the practical utility of this diagnostic approach.

The key findings from this research include: (1) fluorescence-based detection preceded visual symptom appearance by 4.2-7.8 days depending on the specific deficiency; (2) nitrogen deficiency was detected earliest and showed the largest fluorescence changes, consistent with the central role of nitrogen in photosynthetic function; (3) the performance index showed greater sensitivity than F_v/F_m alone, declining by 21-48% before visual symptoms; (4) characteristic fluorescence signatures enabled discrimination among deficiency types with 94.2% classification accuracy; and (5) the F_0/F_m ratio and ψ_0 parameter contributed most to discrimination among deficiencies.

The differential effects of specific nutrient deficiencies on fluorescence parameters reflect the distinct roles of each nutrient in photosynthetic function. Nitrogen deficiency affected multiple components of the photosynthetic apparatus, producing pronounced increases in F_0/F_m indicative of PSII damage. Phosphorus deficiency primarily reduced electron transport efficiency beyond QA, as reflected in decreased ψ_0 values. Iron deficiency produced characteristic modifications to the OJIP transient affecting the I-P phase. These mechanistic differences underlie the ability to discriminate among deficiency types using fluorescence analysis.

The practical implementation of fluorescence-based nutrient diagnosis would require development of standardized measurement protocols, reference databases for different crops and growth stages, and decision support systems for interpreting results and recommending corrective actions. The portable instrumentation used in this research is suitable for field deployment, but factors such as environmental variability and multiple stress interactions would need to be addressed for reliable field applications.

The potential benefits of early nutrient deficiency detection include: reduced yield losses through timely corrective interventions; improved fertilizer use efficiency by targeting applications to areas with demonstrated need; reduced environmental impact through decreased over-application of fertilizers; and enhanced crop quality through maintenance of optimal nutrient status throughout the growing season. These benefits align with the goals of precision agriculture and sustainable intensification.

Future research should evaluate the performance of fluorescence-based diagnosis under field conditions with natural environmental variability and multiple interacting stress factors. Validation across different maize genotypes and growth stages would establish the generalizability of the diagnostic algorithms developed here. Integration with remote sensing platforms could enable large-scale monitoring of crop nutrient status, extending the benefits of early detection to entire production regions.

In conclusion, chlorophyll fluorescence analysis represents a promising approach for early detection of nutrient deficiencies that could enhance the precision and efficiency of crop nutrient management. The non-invasive nature, rapid measurement capability, and diagnostic specificity of this approach offer advantages over traditional diagnostic methods that could translate into improved productivity and environmental outcomes.

References

1. Baker NR. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annu Rev Plant Biol.* 2008;59:89-113.
2. Calatayud A, *et al.* Changes in chlorophyll fluorescence parameters during drought and recovery in pepper plants. *Photosynthetica.* 2006;44(2):303-309.
3. Demmig-Adams B, Adams WW. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends Plant Sci.* 1996;1(1):21-26.
4. Genty B, *et al.* The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta.* 1989;990(1):87-92.
5. Havaux M, Lannoye R. Drought resistance of hard wheat cultivars measured by a rapid chlorophyll fluorescence test. *J Agric Sci.* 1985;104(3):501-504.
6. Krause GH, Weis E. Chlorophyll fluorescence and photosynthesis: the basics. *Annu Rev Plant Physiol.* 1991;42:313-349.
7. Maxwell K, Johnson GN. Chlorophyll fluorescence: a practical guide. *J Exp Bot.* 2000;51(345):659-668.
8. Murchie EH, Lawson T. Chlorophyll fluorescence analysis: a guide to good practice. *J Exp Bot.* 2013;64(13):3983-3998.
9. Percival GC, Fraser GA. Measurement of the salinity tolerance of plants using chlorophyll fluorescence. *Agronomie.* 2001;21(6-7):731-739.
10. Roháček K. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica.* 2002;40(1):13-29.
11. Schreiber U, *et al.* Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching. *Photosynth Res.* 1986;10(1-2):51-62.
12. Strasser RJ, *et al.* Analysis of the chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govindjee, editors. *Chlorophyll *a* Fluorescence*. Dordrecht: Springer; 2004. p. 321-362.
13. van Kooten O, Snel JFH. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth Res.* 1990;25(3):147-150.
14. Živčák M, *et al.* Photosynthetic responses of sun- and shade-grown barley leaves to high light. *Photosynthetica.* 2008;46(2):227-235.
15. Živčák M, *et al.* Application of chlorophyll fluorescence performance indices to assess the wheat photosynthetic function. In: *Photosynthesis Research for Food, Fuel and the Future*. Dordrecht: Springer; 2014. p. 591-599.