

Article

Photosynthetic Performance and Yield Losses of Winter Rapeseed (*Brassica napus* L. var. *napus*) Caused by Simulated Hail

Piotr Dąbrowski ^{1,*} , Łukasz Jełowicki ², Zuzanna M. Jaszczuk ³ , Olena Kryvoviaz ³ and Hazem M. Kalaji ^{4,*} 

¹ Department of Environmental Management, Institute of Environmental Engineering, Warsaw University of Life Sciences—SGGW, Nowoursynowska St. 159, 02-787 Warsaw, Poland

² OPEGIEKA Sp. z o.o., Aleja Tysiąclecia 11, 82-300 Elbląg, Poland; lukasz.jelowicki@opegieka.pl

³ Faculty of Agriculture and Ecology, Warsaw University of Life Sciences—SGGW, Nowoursynowska St. 159, 02-787 Warsaw, Poland

⁴ Department of Plant Physiology, Institute of Biology, Warsaw University of Life Sciences—SGGW, Nowoursynowska St. 159, 02-787 Warsaw, Poland

* Correspondence: piotr_dabrowski@sggw.edu.pl (P.D.); hazem@kalaji.pl (H.M.K.)

Abstract: Winter oilseed rape (*Brassica napus* L.), Europe’s foremost oilseed crop, is significantly impacted by hailstorms, leading to substantial yield reductions that are difficult to predict and measure using conventional methods. This research aimed to assess the effectiveness of photosynthetic efficiency analysis for predicting yield loss in winter rapeseed subjected to hail exposure. The aim was to pinpoint the chlorophyll fluorescence parameters most affected by hail stress and identify those that could act as non-invasive biomarkers of yield loss. The study was conducted in partially controlled conditions (greenhouse). Stress was induced in the plants by firing plastic balls with a 6 mm diameter at them using a pneumatic device, which launched the projectiles at speeds of several tens of meters per second. Measurements of both continuous-excitation and pulse-modulated-amplitude chlorophyll fluorescence were engaged to highlight the sensitivity of the induction curve and related parameters to hail stress. Our research uncovered that some parameters such as F_s , F_m' , Φ_{PSII} , ETR, F_o , F_v/F_m , and F_v/F_o measured eight days after the application of stress had a strong correlation with final yield, thus laying the groundwork for the creation of new practical protocols in agriculture and the insurance industry to accurately forecast damage to rapeseed crops due to hail stress.

Keywords: abiotic stresses; bioindicator; chlorophyll *a* fluorescence; gas exchange; pulse-modulated amplitude fluorescence; JIP-test



Citation: Dąbrowski, P.; Jełowicki, Ł.; Jaszczuk, Z.M.; Kryvoviaz, O.; Kalaji, H.M. Photosynthetic Performance and Yield Losses of Winter Rapeseed (*Brassica napus* L. var. *napus*) Caused by Simulated Hail. *Plants* **2024**, *13*, 1785. <https://doi.org/10.3390/plants13131785>

Academic Editors: Georgia Ouzounidou and Michael Moustakas

Received: 16 May 2024
Revised: 24 June 2024
Accepted: 24 June 2024
Published: 27 June 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Winter rapeseed (*Brassica napus* L., Brassicaceae) stands as a pivotal crop within cereal-dominant crop rotations across European agricultural landscapes. It is prized for its seeds, which are processed into a versatile oil catering to human nutrition, industrial applications, and biodiesel production. Additionally, its by-products serve as valuable animal feed [1]. Among biofuel sources, it boasts the highest energy potential for biodiesel in Europe, particularly the canola variety known for its low erucic acid and glucosinolate content [2].

In recent decades, the escalation of adverse weather events, alongside consequent economic losses, has become more apparent. The agricultural sector’s vulnerability to climate change is increasingly recognized [3]. The World Meteorological Organization (WMO) described hail as a solid form of precipitation, varying from transparent to opaque and composed of ice particles [4]. These can take spherical, conical, or irregular shapes, typically measuring 5 to 50 mm in diameter. Hail formation is influenced by surface conditions and atmospheric circulation, with its development spurred by localized surface features that promote thermal convection and air turbulence during the passage of cold fronts over superheated surfaces due to specific meteorological conditions [4]. The extent

of plant damage from hail depends on various factors including hail size, storm duration, wind speed, crop type, plant growth stage, maturity level, and remaining harvest time [5]. June and July mark the peak hail frequency in Europe, aligning with critical growth stages (BBCH 59 to 69) in crops like rapeseed, where blooms are formed or pod formation is underway [6], making them highly susceptible to mechanical stress and potential agricultural productivity losses. Additionally, the annual frequency of hail events has risen due to climatic changes [4]. Farmers are advised to secure agricultural insurance policies to safeguard against economic losses. Assessing damages after hailstorms involves field investigations by insurance adjusters, using interpretation of photo and professional judgment to identify hail damage thresholds across plant species. Accurate, rapid prediction of yield loss post-hailstorm is vital for setting insurance compensation standards and guiding farmers in post-damage management [7,8].

It is important to note that physiological changes in plants due to hail precede morphological changes, with photosynthesis being particularly vulnerable to hail-induced damage [7]. Photosynthetic activity, especially within Photosystem II (PSII)—the most stress-sensitive component of the photosynthetic machinery—plays a critical role in the plant's response to environmental stressors. Chlorophyll fluorescence (ChF) kinetics analysis emerges as a potent tool for gauging the impact of stress on photosynthesis, offering a reliable assessment of photochemical efficiency. This analysis is crucial for understanding the dynamics of the electron transfer process in photosynthesis, shedding light on the effects of environmental stress on photosynthetic performance [9,10]. There are two main techniques for measuring ChF signals: continuous-excitation chlorophyll fluorescence and pulse-modulated amplitude (PAM) fluorescence. The first technique necessitates a preliminary dark adaptation period of about 20 min for the leaf before measurement can be taken. The JIP-test, a widely used analytical procedure developed by Strasser et al. in 2004 [11], assesses the functionality of Photosystem II (PSII) and its sensitivity to various stresses. Conversely, the PAM fluorescence method does not require a preliminary dark adaptation of the leaf samples because it uses a modulated light source to induce chlorophyll fluorescence. The actinic light is toggled on and off at programmed frequencies, allowing for the measurement of variable components of the induced fluorescence. This facilitates a comprehensive analysis of the dynamic changes in chlorophyll fluorescence. A major advantage of this method is its ability to conduct measurements in natural sunlight, significantly enhancing the applicability of PAM fluorescence for evaluating photosynthetic activity in authentic environmental conditions, as noted by [12].

To address the gap in the literature regarding the estimation of yield losses in winter rapeseed due to hail, there is a notable absence of comprehensive data on utilizing parameters measured through both continuous-excitation and pulse-amplitude modulated (PAM) fluorescence techniques simultaneously. Consequently, this study aimed to pinpoint the chlorophyll fluorescence (ChF) characteristics most vulnerable to hail damage, proposing their use as bioindicators for predicting yield losses in winter rapeseed exposed to hail stress. Identifying these key variables enables the study to achieve two main objectives: first, to elucidate the response of the photosynthetic apparatus in rapeseed to hail stress, thereby providing insights into the crop's adaptability and resilience under such adverse conditions, and second, to underscore significant chlorophyll fluorescence parameters that could aid in the prediction of yield losses, offering a novel approach to assessing the impact of hail on crop productivity.

2. Results

2.1. Yield Losses

The yield of winter oilseed rape was influenced by hail stress (Table 1). Under control conditions, the yield was 3.84 t ha^{-1} . Exposure to hail led to a significant decrease in yield by 53.9%.

Table 1. The yield of winter oilseed rape under hail stress ($\text{t ha}^{-1} \pm \text{S.D.}$). The means marked by the same letter do not differ significantly ($p < 0.05$, $n = 16$).

Treatment	Yield		% Yield Loss
	Mean (t ha^{-1})	$\pm \text{S.D.}$	
Control	3.84 ^a	0.09	0.0
Hail	1.77 ^b	0.07	53.9

2.2. Gas Exchange and Chlorophyll Content Index Results

It was observed that all of the measured parameters were sensitive to hail stress (Table 2). CO_2 assimilation (A_n) in a non-stressed plant was $32.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. Hail caused a reduction by 32.2%. Stomatal conductance (g_s) in non-stressed plants was $0.34 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and it was reduced by hail by 47.1%. Substomatal CO_2 concentration in non-stressed plants was $238 (\mu\text{mol CO}_2 \text{ mol}^{-1})$, and hail caused an increase in its values by 47.5%. Transpiration rate (E) in the control plant was $17.1 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ and was reduced by hail by 48%. Chlorophyll content index was reduced by hail by 36.4% in comparison to the control.

Table 2. The dependence of gas exchange parameters and chlorophyll content index of the winter oilseed rape after the application hail stress: control. A_n : CO_2 assimilation ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); g_s : stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$); E : transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); C_i : substomatal CO_2 concentration ($\mu\text{mol CO}_2 \text{ mol}^{-1}$); CCI: chlorophyll content index (a.u.). The means marked by the same letter indicate no significant difference ($p < 0.05$, $n = 16$).

Treatment	A_n ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)		g_s ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		C_i ($\mu\text{mol CO}_2 \text{ mol}^{-1}$)		E ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)		CCI (a.u.)	
	Mean	$\pm \text{S.D.}$	Mean	$\pm \text{S.D.}$	Mean	$\pm \text{S.D.}$	Mean	$\pm \text{S.D.}$	Mean	$\pm \text{S.D.}$
Control	32.2 ^a	3.0	0.34 ^a	0.04	238 ^b	27	17.1	2.1	22.3 ^a	2.9
Hail	22.5 ^b	1.7	0.18 ^b	0.02	351 ^a	26	8.9	1.4	14.2 ^b	1.5

2.3. JIP-Test Measurements

The course of the OJIP curve was significantly influenced by hail stress (Figure 1). At the J point of the curve, a decrease was observed in comparison to the control. After the normalization procedure, changes were also evident at point O (increase in ChFl value compared to the control) and at point P (decrease in ChFl value compared to the control).

Some parameters of the JIP test were sensitive to hail stress (Figure 2). Among the parameters that significantly increased their values under the influence of stress were minimal fluorescence (F_0), electron transport flux per RC (ET_0/RC), efficiency/probability that an electron moves further than Q_A^- (ψ_{E0}), and efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (δ_{R0}). On the other hand, among the parameters that significantly decreased their values were F_v/F_0 , V_J , performance indexes (PI_{abs} and PI_{tot}), and relative driving forces (DF_{abs} and DF_{tot}).

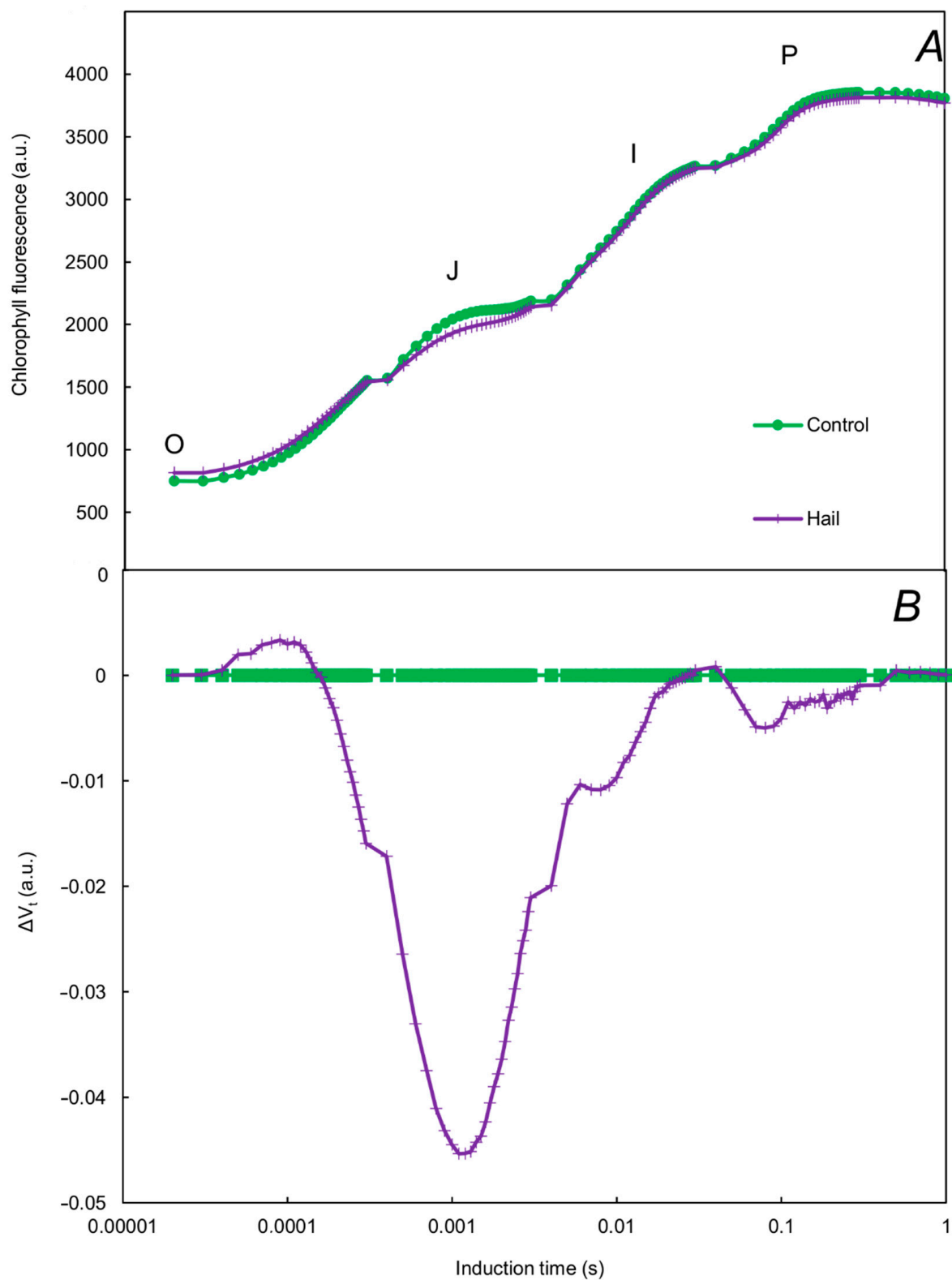


Figure 1. Induction curves of chlorophyll *a* fluorescence (A) and differential curves of ΔV_t (obtained by subtracting the control curve from the first sample) (B) of winter oilseed rape under the influence of hail.

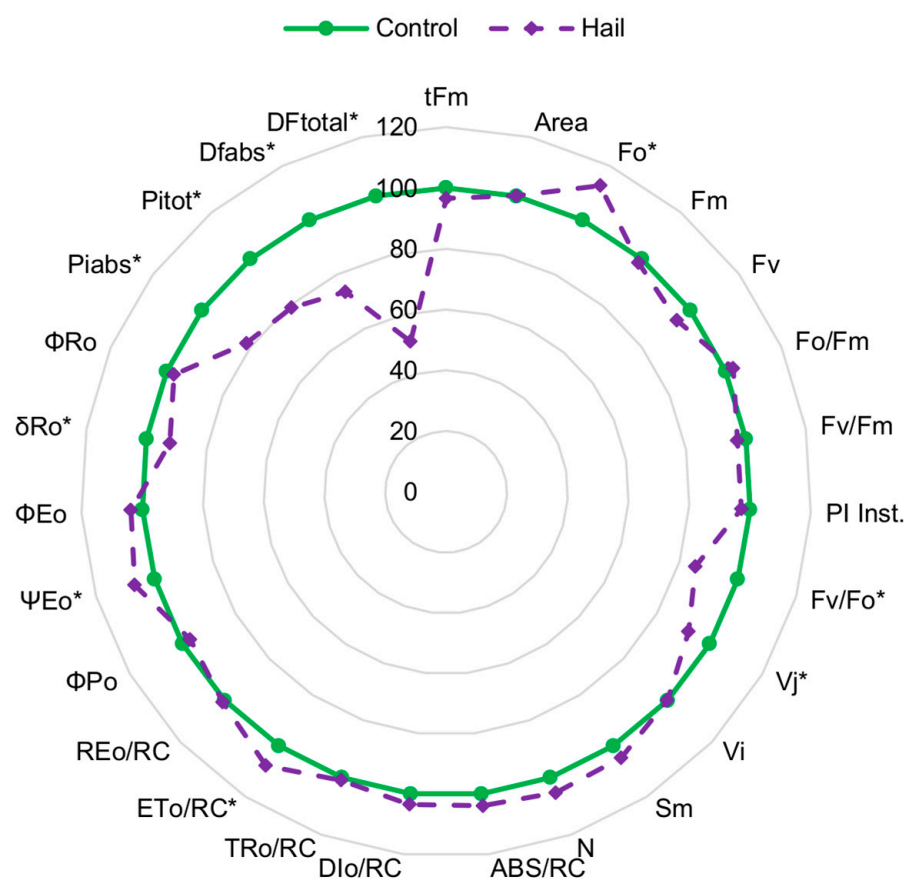


Figure 2. The JIP test parameters normalized to the values before stress application (control) as radar plots of winter rapeseed under the influence of hail (a.u. $\pm$ S.D.). Means within particular parameters marked by the asterisk differed significantly from the control ($p < 0.05$, $n = 16$). Time to reach the maximal fluorescence (tF_m), area above the OJIP curve (Area), minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence (F_v), ratio of the efficiency of primary photochemical reactions (F_o/F_m), maximum quantum yield of primary photochemical reactions (F_v/F_m), maximum efficiency of the water diffusion reaction (F_v/F_o), relative variable fluorescence at the J and I steps (V_j and V_i), normalized total area above the OJIP curve (S_m), absorption flux per one active RC (ABS/RC), energy flux not intercepted by an RC (DI_o/RC), energy flux trapped by one active RC (TR_o/RC), rate of electron transport by one active RC (ET_o/RC), efficiency index expressed as the density of RCs per chlorophyll (Chl) (RE_o/RC), maximum quantum yield of primary photochemical reactions (ΦP_o), quantum yield for electron transport (ΦE_o), efficiency/probability that an electron moves further than Q_A^- (ΨE_o), efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (δR_o), quantum yield for reduction of end electron acceptors at the PSI acceptor side (ΦR_o), performance indexes (PI_{inst} , PI_{abs} , and PI_{tot}), driving forces (DF_{abs} and DF_{tot}).

2.4. PAM Measurements

All parameters obtained from PAM measurements were significantly influenced by hail stress (Figure 3). Under control conditions, the steady-state fluorescence (F_s) parameter was 773 (a.u.). Exposure to hail led to a significant increase in its value by 112%. Under control conditions, the maximum chlorophyll *a* fluorescence in light-adapted leaves (F_m' parameter) was 3208 (a.u.), and statistical analysis did confirm significant influence of hail on this parameter. Exposure to this stress caused reduction in this parameter by 32.8%. Under control conditions, the estimated effective quantum yield (efficiency) of PSII photochemistry at given PAR (Φ_{PSII} parameter) was 0.75 (a.u.), and statistical analysis did also confirm the influence of hail on this parameter. Exposure to this stress caused a

reduction in this parameter in comparison to the control by 67.7%. The electron transport rate (ETR parameter) also underwent reduction by 71.0% in comparison to the control.

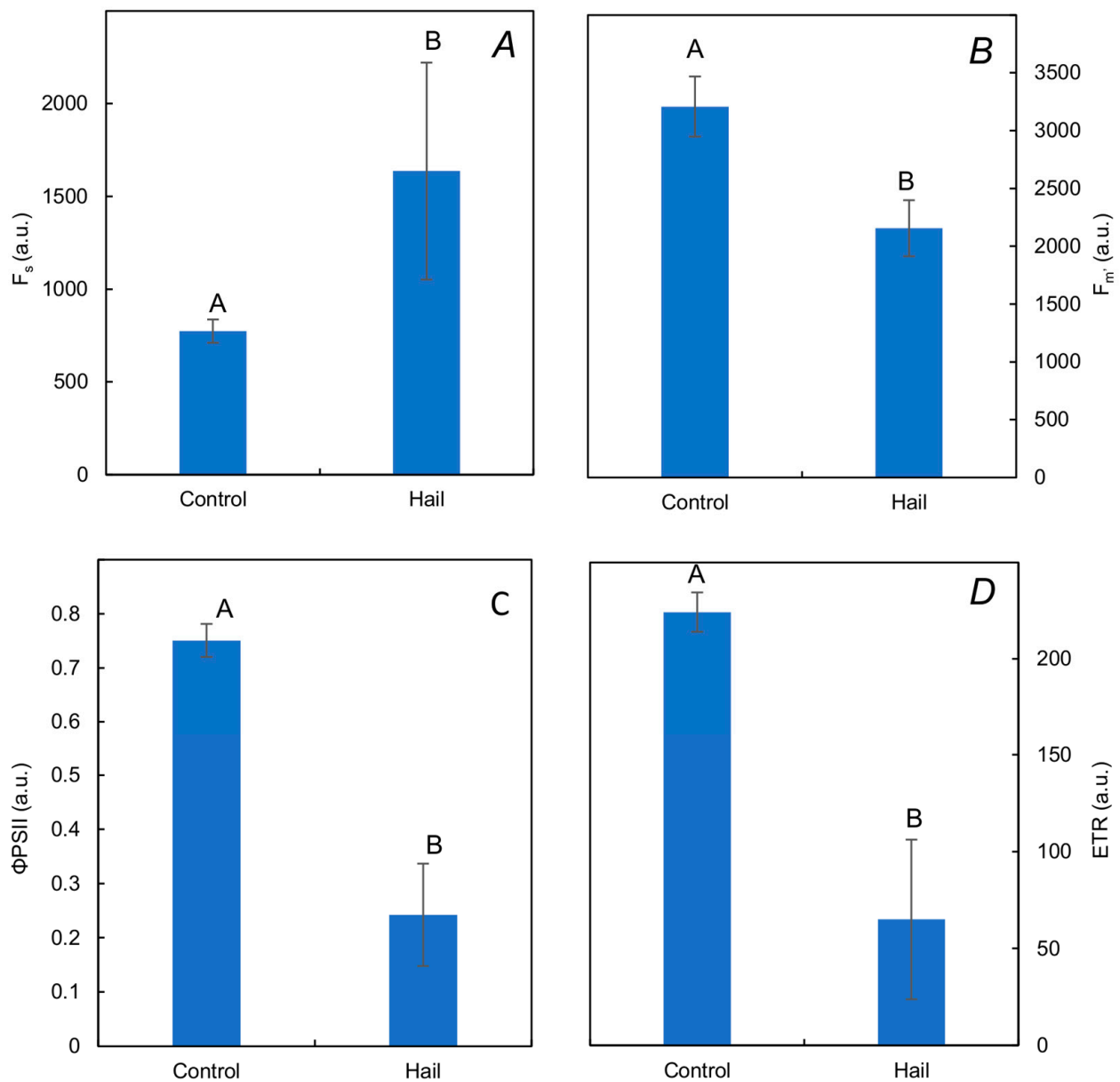


Figure 3. The PAM (pulse-modulated amplitude fluorescence) parameters: steady-state fluorescence at any light level (F_s) (A), maximum fluorescence from light-adapted leaf (F_m') (B), estimated effective quantum yield (efficiency) of PSII photochemistry at given PAR (Φ_{PSII}) (C), and electron transport rate (ETR) (D) of winter rapeseed under the influence of hail: ($t\ ha^{-1} \pm S.D.$). The means marked by the same letter did not differ significantly ($p < 0.05$, $n = 16$).

2.5. Relationship between Hail Stress and Individual Parameters of Photosynthetic Performance

A significant correlation was observed between yield loss and all measured gas exchange parameters (Table 3). Stomatal CO_2 concentration was correlated negatively, and all other parameters (A_n , g_s and E) were correlated positively. Also, chlorophyll content index (CCI) was correlated positively.

Table 3. The Pearson correlation coefficient (r) between the yield of winter rapeseed and individual physiological parameters under the influence of hail. Values marked by an asterisk are significant ($p < 0.05$; $n = 32$). The photosynthetic rate in terms of net CO₂ assimilation (A_n), stomatal conductance (g_s), substomatal CO₂ concentration (C_i), transpiration rate (E), chlorophyll content index (CCI), steady-state fluorescence (F_s), maximal chlorophyll a fluorescence in light-adapted leaves (F_m'), estimated effective quantum yield (efficiency) of PSII photochemistry at given PAR (Φ_{PSII}), electron flow rate (ETR), time to reach the maximal fluorescence (t_{Fm}), area above the OJIP curve (Area), minimal fluorescence (F_o), maximal fluorescence (F_m), variable fluorescence (F_v), ratio of the efficiency of primary photochemical reactions (F_o/F_m), maximum quantum yield of primary photochemical reactions (F_v/F_m), maximum efficiency of the water diffusion reaction (F_v/F_o), relative variable fluorescence at the J and I steps (V_j and V_i), normalized total area above the OJIP curve (S_m), absorption flux per one active RC (ABS/RC), energy flux not intercepted by an RC (DI_o/RC), energy flux trapped by one active RC (TR_o/RC), rate of electron transport by one active RC (ET_o/RC), efficiency index expressed as the density of RCs per chlorophyll (Chl) (RE_o/RC), maximum quantum yield of primary photochemical reactions (ϕ_{Po}), quantum yield for electron transport (ϕ_{Eo}), efficiency/probability that an electron moves further than Q_A^- (ψ_{Eo}), efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (δ_{Ro}), quantum yield for reduction of end electron acceptors at the PSI acceptor side (ϕ_{Ro}), performance indexes (PI_{inst} , PI_{abs} , and PI_{tot}), driving forces (DF_{abs} and DF_{tot}).

Parameter	r	Parameter	r	Parameter	r	Parameter	r
A_n	0.86 *	Area	−0.02	N	−0.15	ϕ_{Ro}	0.05
g_s	0.92 *	F_o	−0.77 *	ABS/RC	−0.27	PI_{abs}	0.41 *
C_i	−0.90 *	F_m	0.22	DI_o/RC	−0.11	PI_{total}	0.37 *
E	0.92 *	F_v	0.53 *	TR_o/RC	−0.07	DF_{abs}	0.56 *
CCI	0.88 *	F_o/F_m	−0.13	ET_o/RC	−0.52 *	DF_{Total}	0.63 *
F_s	−0.71 *	F_v/F_m	0.74 *	RE_o/RC	−0.03	O	−0.64 *
F_m'	0.74 *	F_v/F_o	0.75 *	ϕ_{Po}	0.74 *	K	−0.12
Φ_{PSII}	0.94 *	V_j	0.49 *	ψ_{Eo}	−0.49 *	J	0.30
ETR	0.95 *	V_i	0.02	ϕ_{Eo}	−0.29	I	0.05
t_{Fm}	0.05	S_m	−0.14	δ_{Ro}	0.18	P	0.14

It was discovered that some specific chlorophyll a fluorescence parameters were significantly correlated with the yield loss attributed to hail stress, as detailed in Table 3 (parameters marked by asterisk). Among the parameters positively correlated were F_m' , Φ_{PSII} , ETR, F_v , F_v/F_m , F_v/F_o , V_j , ϕ_{Po} , PI_{abs} , PI_{tot} , DF_{abs} , and DF_{total} . The values of correlation coefficients of these parameters ranged from 0.38 to 0.95. Among the parameters negatively correlated were F_s , ET_o/RC , ψ_{Eo} , and O point from the OJIP curve. The values of correlation coefficient of these parameters ranged from −0.49 to −0.77.

In the vector graphs (Figure 4A), the relative “contribution” of each input variable to the formation of the principal components (Dim1 and Dim2) is presented. The magnitude of the vector is an indicator of the hail influence on corresponding ChFl parameter, and the vector direction depends on this impact on the Dim1 and Dim2 value. The modifications in the first principal component (Dim1) determined 35.7% of total changes, and the second component Dim2 reflected 32.3%. The analyzed ChFl parameters had different sensitivity to hail stress, as well as different contributions in the formation of principal components. There were groups of parameters, which were positively correlated with yield, such as A_n , g_s , C_i , ETR, Area, F_m , and ABS/RC. On the other hand, there were parameters negatively correlated with yield, such as F_m' , ϕ_{Po} , ϕ_{Eo} , and ψ_{Eo} . It is worth noting that there was the largest group of parameters, for which vectors had 90° to yield vectors. This group can be

Figure 4. Principal component analysis of the salt stress effect on the photosynthetic machinery of winter oilseed rape and its yield under hail stress: vector graphs showing the relative “contribution” of each input variable to the formation of the principal components (**A**); biplot (**B**). CCI (1), A_n (2), g_s (3), C_i (4), E (5) F_s (6), F_m' (7), Φ_{PSII} (8), ETR (9), t_{Fm} (10), Area (11), F_o (12), F_m (13), F_v (14), F_o/F_m (15), F_v/F_m (16), $PI_{inst.}$ (17), F_v/F_o (18), V_j (19), V_i (20), S_m (21), N (22), ABS/RC (23), DI_o/RC (24), TR_o/RC (25), ET_o/RC (26), RE_o/RC (27), ϕ_{Po} (28), ϕ_{Eo} (29), ψ_{Eo} (30), δ_{Ro} (31), ϕ_{Ro} (32), PI_{abs} (33), PI_{tot} (34), DF_{abs} (35), DF_{tot} (36), O point (37), K point (38), J point (39), I point (40), and P point (41).

The majority group of the control and stressed plants of winter rapeseed are presented (Figure 4B). For plants not subjected to hail stress, the values of the principal components varied at around 0 to 5 for Dim1 and -1 to 2 for Dim2. In plants subjected to hail stress, incubation of the physiological state of the photosynthetic apparatus was changed—the population varied at around -8 to 5 for Dim1 and around -18 to 7 for Dim2.

3. Discussion

Literature reports on the impact of hail on plant physiology are limited. Existing studies indicate that photosynthetic efficiency, assessed through gas exchange measurements and the analysis of specific chlorophyll fluorescence (ChFl) parameters, significantly decreases in trees [13,14] and shrubs [15] subjected to this type of mechanical stress. Additionally, a decline in yield was observed in these studies, as well as in potatoes [16].

The primary objective of our research was to investigate whether particular physiological parameters, measured through gas exchange and both continuous-excitation and pulse-amplitude-modulated (PAM) chlorophyll fluorescence techniques, could serve as reliable indicators of hail stress in winter oilseed rape plants. We posited that ChFl measurements might provide a dependable method for detecting hail stress in oilseed rape prior to the manifestation of visible symptoms. This hypothesis is founded on the presumed correlation between ChFl signals and photosynthetic efficiency.

The primary energy source for all autotrophic plants' growth and development is photosynthesis in their leaves. It is now commonly acknowledged that stomatal conductance and/or leaf biochemical capacity are the primary factors limiting photosynthesis in C_3 plants under stress [17]. Our studies confirmed such gas exchange parameters as CO_2 assimilation and H_2O transpiration and stomatal conductance decreased in winter oilseed rape under hail stress. Meanwhile, intercellular CO_2 concentration increased. These results can suggest that CO_2 accumulates within intercellular spaces of the leaf when it is not assimilated by Rubisco [18].

The pivotal discovery of our research was the identification of specific points along the OJIP curve that are sensitive to hail stress. Our findings revealed that the J point of the curve maintained its sensitivity under hail stress conditions. Additionally, the O and P points also showed some sensitivity, as indicated by their values being comparable after subtracting the control curve from the first sample (ΔV_t). Notably, there is a lack of information in existing literature regarding alterations in the shape of the OJIP curve in response to hail stress. Nonetheless, the pattern of sensitivity across the OJIP curve points in plants subjected to various abiotic stresses has been extensively documented [19,20]. Furthermore, modifications in the values of specific points on the curve were also observed in rapeseed plants experiencing temperature stress [21,22], underscoring the potential of these markers in assessing plant response to environmental pressures.

Next, the specific JIP-test parameters were analyzed and presented in a radar plot format. It was proved that only a few parameters were receptive to hail stress. This emphasizes that mechanical stress has a complex effect on the physiological status of winter oilseed rape and highlights how important it is to use modern fluorescence techniques to identify and measure stress responses. This phenomenon can be followed well based on the values of the performance index parameters (PI_{abs} and PI_{tot}) and driving forces (DF_{abs} and DF_{tot}), which were significantly changed by this stress. The sensitivity of those parameters on low-temperature stresses in winter oil seed rape was confirmed by [21–23]. The sensitivity of those parameters was also confirmed in other plant species under different abiotic stresses [24,25]. On the other hand, the F_v/F_m parameter was significantly decreased by simulated hail in apple trees [14] and in grapes [26]. Our research did not confirm a significant decrease in the F_v/F_m parameter in winter oil rapeseed under hail stress; however, there was a significant correlation between its values and yield. Moreover, as in the studies of [14,26], we confirmed the increase in the F_o parameter caused by this stress.

Hail stress caused significant modifications in all four PAM parameters measured in light-adapted samples illuminated with actinic light. These parameters are important for

understanding the molecular dynamics of photosynthesis under various biotic and abiotic stresses [9,27]. Many researchers are certain that PAM analysis is more intricate than OJIP analysis since it integrates both photochemical and non-photochemical quenching [28,29].

Our research proved that F_s (a parameter also known as steady-state fluorescence, which reflects the relative fluorescence intensity) is sensitive to hail stress. The increase in this parameter can be interpreted as an imbalance between the rates of ATP, NADPH synthesis, and CO_2 fixation [12]. In such a situation, the Calvin–Benson cycle devours less ATP and NADPH for CO_2 fixation than is generated by the primary processes of photochemical photosynthesis [30]. As a consequence, the quantum efficiency of PSII photochemistry is reduced. The increase in this parameter can also indicate that some PSII reaction centers are unable to utilize excitation energy effectively under light conditions, which led to an increase in non-photochemical de-excitation (NPQ) [31].

The F_m' parameter was significantly decreased by hail stress. Moreover, there was a significant correlation between its values and the yield of winter oil rapeseeds. In general, fluctuation of this parameter reflects changes in the rate constant of regulated non-photochemical quenching. The changes in its values might express the extent of the regulated loss of non-photochemical energy [32].

The decrease in the Φ_{PSII} parameter in winter oil rapeseed under hail was noted in our research. It should be underlined that our research confirms the highest relationship of this parameter (among all tested ChFl parameters) and yield loss. The correlation coefficient (r) between these parameters was 0.94. This parameter represents the fraction of the light energy absorbed by PSII, which drives photosynthetic electron transport [12]. It is frequently used in field research and might be interpreted as the effective quantum yield of the PSII photochemistry related to the actual fraction of photochemically active PSII RCs (qP) [33].

ETR represents the rate of photosynthetic processes in plant samples and correlates well with the quantum yield of CO_2 assimilation and stomatal conductance [12]. The decrease in the ETR parameter in winter oil rapeseed under hail was noted in our research. This phenomenon was found in apple trees [14] and in grapes [28].

A multi-parametric analysis was used to evaluate the stress effects in winter rapeseed in order to identify parameters that are most sensitive to the plant stress response. The separate parameters are not fully autonomous (both in the case of the JIP-test parameters and PAM parameters) because some of them are calculated on the basis of points of the chlorophyll fluorescence curve [34]. Some of them are connected by mathematical expressions (e.g., φ_{P_0} and φ_{D_0}). Plant stress response is accurately described by each of the ChFl parameters, yet each parameter represents a different characteristic of photosynthetic machinery. Although the comparison of the samples was overly complicated, the multiparametric description based on the constellation of factors provides a more detailed explanation of plant photosynthesis. Principal component analysis (PCA), which enables a better understanding of the stressor effect on the photosynthetic machinery as a whole, is an efficient way to employ such a set of experimental characteristics [35]. By utilizing the PCA-based technique, we are able to fully utilize the fluorescence data and derive new features that accurately depict the response of plant photosynthetic processes to stress. The stress-induced changes in the plants under investigation were displayed in Figure 4 as a 2D graph on a plane with Cartesian coordinates labeled “Dim1” and “Dim2” for easier visualization.

Finally, we concluded that the calculation of crop production losses due to hail stress can be facilitated by employing measurements of plant photosynthetic efficiency to track changes in the winter oilseed rape. Our research revealed that some of chlorophyll fluorescence parameters such as F_s , F_m' , Φ_{PSII} , ETR, F_0 , F_v/F_m , and F_v/F_0 measured directly after stress application have a strong correlation with final yield, thus laying the groundwork for the creation of new practical protocols in agriculture and insurance industry to accurately forecast damage to rapeseed crops due to hail stress. Our research improved our knowledge of how vulnerable crops are to the sudden hailstorms. However, this paper reports on a

study that was limited to a single crop variety and a one-year experiment that was carried out in partially controlled conditions. Future studies should be conducted in the field on a larger number of crop varieties and experimental seasons to expand on these insights and support our findings. Moreover, our research did not set out to directly compare the performance of different algorithms in forecasting crop yield losses. Existing literature indicates that nonlinear algorithms, such as random forests, support vector machines, and artificial neural networks, might be more effective than linear algorithms in processing chlorophyll fluorescence data to predict yield losses.

4. Materials and Methods

4.1. Experimental Design and Plant Growth Conditions

The experiment was carried out in semi-controlled conditions in an open vegetation hall (open greenhouse) at Warsaw University of Life Sciences (WULS). Winter rapeseed plants (variety LG Areti, Limagrain, Saint Beaulieu, France,) were sown in 32 pots (5 seeds per pot) on 12 September 2022. Each pot contained 6 kg of substrate composed of 60% peat, 30% composted bark, and 10% sand. After preparing the mixture, 11 kg m⁻³ of chalk was added (to reach pH 6), followed by MIS4 fertilizer + microelements (120 g m⁻³) (Intermag, Olkusz, Poland). On 2 October, 0.5% calcium nitrate + folium 0.6% + L amino H 0.6% was sprayed. On 5 October, 3% MgSO⁴⁺ Radiculum 0.6% was sprayed.

The outside section of the greenhouse was then used to house all of the pots, creating a natural environment. At this time, no new stressors were added. The most significant weather conditions for the experiment are listed in Table 4.

Table 4. Meteorological conditions prevailing in the outside of the greenhouse during the experiment.

Month	Average Month Temp. [°C]		Min. Month Temp. [°C]		Max. Month Temp. [°C]		Precipitation [mm]		Solar Radiation [W m ⁻²]	
	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
September	12.6	-	3.2	-	17.2	-	56.0	-	160.3	-
October	11.6	-	0.2	-	16.2	-	31.4	-	132.3	-
November	4.4	-	-6.5	-	6.6	-	22.1	-	48.1	-
December	0.8	-	-13.3	-	2.6	-	61.6	-	29.4	-
January	-	3.6	-	-1.5	-	18.7	-	62.3	-	28.5
February	-	1.8	-	-7.7	-	10.0	-	41.2	-	59.8
March	-	4.9	-	-5.4	-	19.4	-	26.7	-	111.0
April	-	9.4	-	-3.4	-	22.9	-	57.2	-	176.1

Plants that had been dormant during the winter broke their hibernation on 29 January 2023, when all of the pots were moved into a closed interior area of the greenhouse. During this time, the average daily temperature was about 18 °C, and HPSC lamps were used to provide additional lighting for the plants (200 µmol m⁻² s⁻¹).

On 20 April 2023, the rape plants were in phase BBCH 65–67 (full flowering: 50% of the flowers on the main inflorescence are open, older petals fall off) [6]. At this time, pots were divided randomly into 2 groups (16 pots per each group). Each group of vases constituted a separate research variant. The variants are presented below:

- (i) Control;
- (ii) Hail stress.

Hail stress was simulated by firing at plants with a pneumatic device that launched 6 mm diameter plastic balls at speeds of several tens of meters per second. The firing was carried out from an elevated position (distance of a few meters) to mimic hail falling from above, ensuring each plant received an identical number of impacts. All measurements of photosynthetic efficiency were taken on 28 April 2023. Subsequently, the plants were

grown under natural conditions in the open section of the greenhouse until the end of the vegetation period. The harvest was collected on 7 July 2023.

The measurements of chlorophyll fluorescence, greenness index, and gas exchange were always performed on the same part of the chosen leaf: halfway along its length and one-quarter of its width. Leaves were selected from the middle canopy layers. In the case of non-stressed plants (control), measurements were conducted on intact leaves. However, in the case of plants treated with hail, only damaged leaves were measured, avoiding close spots of the hail damage.

Stress was applied on 20 April, when the rape plants were in phase BBCH 65–67 (full flowering: 50% of the flowers on the main inflorescence are open, older petals fall off). This term was chosen because, at this growth stage, the occurrence of hail causes the greatest losses. The measurements were performed on 28 April, so 8 days after stress application.

4.2. Yield Measurements

Following the photosynthetic performance measurement, the plants underwent a natural desiccation process. Afterwards, seeds were harvested separately from each pot and weighed to determine the actual yield. The results obtained were then converted to $t\ h^{-1}$.

4.3. Gas Exchange Measurements and Chlorophyll Content Index Measurement

One plant from each pot was selected at random. To improve the correlation index between gas exchange, chlorophyll content index, and ChFI signals, all the measurements were made on previously marked leaves. On each plant, one leaf was selected for the measurement. Measurements were always performed on the same part of the leaf: halfway along its length and one-quarter of its width. Leaves were selected from the middle tiers, specifically the fourth or fifth tier. The photosynthetic rate in terms of net CO_2 assimilation (A_n), stomatal conductance (g_s), transpiration rate (E), and the substomatal CO_2 concentration (C_i) were all measured on this same leaves as ChFI and CCI using a portable gas analyzer Lcpro⁺ (ADC BioScientific Ltd., Hoddesdon, UK). This open-gas exchange system operated during measurement on a differential mode at a $150\ mol\ s^{-1}$ flow rate of ambient air. The measurements were taken after the stabilization of conditions in the chamber.

The chlorophyll content index (CCI) measurements were made by using a CCM-200 (Opti-Sciences, Inc., Hudson, NH, USA) chlorophyll content meter.

4.4. Chlorophyll *a* Fluorescence Measurement

Chlorophyll *a* fluorescence was assessed using two fluorimeters:

- HandyPEA (Hansatech Instruments Ltd., King's Lynn, UK);
- FMS-2 (Hansatech Instruments Ltd., King's Lynn, UK).

To better visualize the influence of the stress on the dynamics of the chlorophyll transients, the relative variable fluorescence intensity (V_t) was calculated. At the next stage, the differences of relative variable fluorescence intensity (ΔV_t) were calculated by subtracting the normalized fluorescence values (between O and P steps) recorded in control plants and under stress. V_t and ΔV_t were calculated according to the formulas:

$$V_t = (F_t - F_o) / (F_m - F_o) \quad (1)$$

$$\Delta V_{t, \text{stress}} = V_{t, \text{stress}} - V_{t, \text{control}} \quad (2)$$

All chlorophyll fluorescence parameters measured by HandyPEA after dark adaptation (JIP test) are presented in Table 5.

Table 5. Glossary, definition of terms, and formulae used by the JIP-test for the analysis of the Chl *a* fluorescence transient OJIP emitted by dark-adapted photosynthetic samples ([36]).

t_{Fm}	time (in ms) to reach the maximal fluorescence FP (meaningful only when $F_P = F_m$)
Area	total complementary area between the fluorescence induction curve and $F = FP$ (meaningful only when $F_P = F_m$)
$F_o \cong F50 \mu s$ or $\cong F20 \mu s$	fluorescence when all PSII RCs are open ($\cong$ to the minimal reliable recorded fluorescence)
F_m	maximal fluorescence, when all PSII RCs are closed
$F_v \equiv F_m - F_o$	maximal variable fluorescence
F_v/F_m	maximum quantum yield for primary photochemistry
$ABS/RC = M_o \times (1/V_j) \times (1/\phi_{Po})$	absorption flux (exciting PSII antenna Chl <i>a</i> molecules) per RC (also used as a unit-less measure of PSII apparent antenna size)
$TR_o/RC = M_o \times (1/V_j)$	trapped energy flux (leading to Q_A reduction), per RC
$RE_o/RC = M_o \times (1/V_j) \times (1 - V_I)$	electron flux reducing end electron acceptors at the PSI acceptor side, per RC
$ET_o/RC = M_o \times (1/V_j) \times (1 - V_j)$	electron transport flux (further than Q_A^-), per RC
$\phi_{Po} \equiv TR_o/ABS = [1 - (F_o/F_m)]$	maximum quantum yield for primary photochemistry
$\phi_{Eo} \equiv ET_o/ABS = [1 - (F_o/F_m)] \times (1 - V_j)$	quantum yield for electron transport (ET)
$\phi_{Ro} \equiv RE_o/ABS = [1 - (F_o/F_m)] \times (1 - V_I)$	quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\psi_{Eo} \equiv ET_o/TR_o = (1 - V_j)$	efficiency/probability that an electron moves further than Q_A^-
$\delta_{Ro} \equiv RE_o/ET_o = (1 - V_I)/(1 - V_j)$	efficiency/probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (RE)
$N = S_m \times (M_o/V_j)$	turnover number (expresses how many times Q_A is reduced in the time interval from 0 to t_{FM})
PI_{abs}	performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors
PI_{total}	total performance index for energy conservation from photons absorbed by PSII until the reduction of PSI end electron acceptors

Before the measurements, the middle section of each leaf was dark adapted for at least 25 min by using Hansatech leaf clips. A brief non-actinic light flash was applied to the leaf just before recording the fluorescence transients to adjust the detector gain. Subsequently, each leaf sample was exposed to continuous saturating actinic light ($3500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). PAM measurements conducted by the FMS-2 fluorimeter were performed in close proximity to the clips immediately following continuous-excitation chlorophyll fluorescence.

An FMS-2 fluorimeter (Hansatech Instruments Ltd., King's Lynn, UK) was used for the PAM (pulse-modulated amplitude fluorescence) measurements after adaptation of the plants to light. The following were the 4 measured parameters:

- F_s —Steady-state fluorescence at any light level. This parameter indicates the intensity of chlorophyll fluorescence, which accompanies the photosynthesis process in stationary conditions.
- F_m' —Maximum chlorophyll *a* fluorescence in light-adapted leaves.
- Φ_{PSII} (yield or Genty parameter)—Estimated effective quantum yield (efficiency) of PSII photochemistry at a given PAR. Based on changes in the values of this parameter, the quantum yield of the photochemical reaction in PSII can be assessed.
- ETR—electron flow rate through photosystems.

We used the following protocol for PAM measurements:

1. By the use of special leaf clips, plants were adapted to darkness for about 15 min;
2. First pulse light ($4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was activated for 1 s (F_o and F_m measured);

3. Wait until signal gets to a steady state;
4. Actinic light ($1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was activated (F_p measured);
5. Wait 4–5 min until the signal gets to a steady state (a time that is enough for full stomata adaptation and achieving equilibrium between light and dark phases of photosynthesis) (F_s was measured);
6. Second pulse light ($12,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was activated for 1 s (F_m' measured).

4.5. Statistical Analysis

There were 16 ChFl measurements in each treatment ($n = 16$). Student's t-test at a 0.05 confidence level was used to analyze the obtained parameters and yield. The mathematical relationship between chlorophyll fluorescence signals, and yield losses were estimated based on Pearson's correlation coefficient at a 0.05 confidence level. Statistica 10.0 program (Statsoft, Inc., Tulsa, OK, USA) was used to perform all statistical analyses.

Author Contributions: Conceptualization, P.D. and H.M.K.; methodology, P.D. and Ł.J.; formal analysis, P.D. and Ł.J.; investigation, P.D., Ł.J. and H.M.K.; data curation, Z.M.J. and O.K.; writing—original draft preparation, P.D.; writing—review and editing, Ł.J. and H.M.K.; visualization, P.D.; supervision, P.D.; P.D., 70%; Ł.J., 10%; Z.M.J., 5%; O.K., 5%; H.M.K., 10%. All authors have read and agreed to the published version of the manuscript.

Funding: The article was developed as part of the project titled “InsFlo—Fluorescence of crops in estimating losses caused by weather factors”, implemented by OPEGIEKA Sp. z o.o., co-financed by the European Union from the European Regional Development Fund under the Smart Growth Operational Programme 2014–2020, Priority Axis I “Support for R&D activity of enterprises”, Measure 1.1 “R&D projects of enterprises”, Sub-measure 1.1.1 “Industrial research and development work implemented by enterprises”.

Data Availability Statement: The data published in this article are available from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Hausmann, J. Challenges for integrated pest management of *Dasineura brassicae* in oilseed rape. *Arthropod-Plant Interact.* **2021**, *15*, 645–656. [\[CrossRef\]](#)
2. Jankowski, K.J.; Sokólski, M.; Załuski, D. Winter oilseed rape: Agronomic management in different tillage systems and energy balance. *Energy* **2023**, *277*, 127590. [\[CrossRef\]](#)
3. Rusteika, M.; Skinulienė, L. Expectations of the Participants of the Crop Insurance System and Their Implementation. *Agriculture* **2023**, *13*, 649. [\[CrossRef\]](#)
4. WMO (World Meteorological Organization). *Guidelines on the Definition and Monitoring of Extreme Weather and Climate Events. Task Team Defin Extrem Weather clim Events*; WMO: Geneva, Switzerland, 2018.
5. Felix, M.O.; Kennedy, P. Correlation of polarimetric radar hail signature with MODIS satellite ground truth data. In Proceedings of the 87th AMS Annual Meeting, San Antonio, TX, USA, 14–18 January 2007.
6. Lorenz, H.; Kabelitz, R.; Bleiholder, H.; Hofmann, M. Phänologische Entwicklungsstadien von Gemüsepflanzen: II. Fruchtgemüse und Hülsenfrüchte. *Nachrichtenblatt Dtsch. Pflanzenschutz.* **1994**, *46*, 217–232.
7. Wang, L.; Liu, L.; Wen, M.; Li, M.; Dong, Z.; He, Z.; Cui, J.; Ma, F. Using field hyperspectral data to predict cotton yield reduction after hail damage. *Comput. Electron. Agric.* **2021**, *190*, 106400. [\[CrossRef\]](#)
8. Angearu, C.-V.; Ontel, I.; Irimescu, A.; Sorin, B.; Dodd, E. Remote sensing methods for detecting and mapping hailstorm damage: A case study from the 20 July 2020 hailstorm, Baragan Plain, Romania. *Nat. Hazards* **2022**, *114*, 2013–2040. [\[CrossRef\]](#)
9. Baker, N.R.; Rosenqvist, E. Applications of chlorophyll fluorescence can improve crop production strategies: An examination of future possibilities. *J. Exp. Bot.* **2004**, *55*, 1607–1621. [\[CrossRef\]](#)
10. Kalaji, H.M.; Schansker, G.; Brestic, M.; Bussotti, F.; Calatayud, A.; Ferroni, L.; Goltsev, V.; Guidi, L.; Jajoo, A.; Li, P.; et al. Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynth. Res.* **2017**, *132*, 13–66. [\[CrossRef\]](#)
11. Strasser, R.; Tsimilli-Michael, M.; Srivastava, A. Analysis of the Chlorophyll a Fluorescence Transient. In *Chlorophyll a Fluorescence: A Signature of Photosynthesis*; Papageorgiou, Govindjee, G.C., Eds.; Springer: Dordrecht, The Netherlands, 2004; pp. 321–362. [\[CrossRef\]](#)

12. Kalaji, H.M.; Goltsev, V.N.; Zuk-Golaszewska, K.; Zivcak, M. *Chlorophyll Fluorescence: Understanding Crop Performance: Basics and Applications*; CRC Press: Boca Raton, FL, USA, 2017.
13. Tartachnyk, I.; Blanke, M.M. Effect of mechanically-simulated hail on photosynthesis, dark respiration and transpiration of apple leaves. *Environ. Exp. Bot.* **2002**, *48*, 169–175. [\[CrossRef\]](#)
14. Tartachnyk, I.; Blanke, M.M.; Jackson, M.B. Effect of hail on photosynthesis, chlorophyll fluorescence, stomatal closure and evapotranspiration of apple leaves. *Acta Hort.* **2007**, *732*, 543–547. [\[CrossRef\]](#)
15. Petoumenou, D.G.; Biniari, K.; Xyrafis, E.; Mavronasios, D.; Daskalakis, I.; Palliotti, A. Effects of Natural Hail on the Growth, Physiological Characteristics, Yield, and Quality of *Vitis vinifera* L. cv. Thompson Seedless under Mediterranean Growing Conditions. *Agronomy* **2019**, *9*, 197. [\[CrossRef\]](#)
16. Jalali, A.H. Potato (*Solanum tuberosum* L.) yield response to simulated hail damage. *Arch. Agron. Soil Sci.* **2013**, *59*, 981–987. [\[CrossRef\]](#)
17. Flexas, J.; Carriqui, M. Photosynthesis and photosynthetic efficiencies along the terrestrial plant's phylogeny: Lessons for improving crop photosynthesis. *Plant J.* **2020**, *101*, 964–978. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Deller, Y.; Jossier, M.; Bouchereau, A.; Hodges, M.; Lepout, L. Leaf Phenological Stages of Winter Oilseed Rape (*Brassica napus* L.) Have Conserved Photosynthetic Efficiencies but Contrasted Intrinsic Water Use Efficiencies at High Light Intensities. *Front. Plant Sci.* **2021**, *12*, 659439. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Dąbrowski, P.; Pawluśkiewicz, B.; Baczeńska, A.H.; Ogłęcki, P.; Kalaji, H. Chlorophyll a fluorescence of perennial ryegrass (*Lolium perenne* L.) varieties under long term exposure to shade. *Zemdirbyste-Agriculture* **2015**, *102*, 305–312. [\[CrossRef\]](#)
20. Dąbrowski, P.; Keutgen, A.J.; Keutgen, N.; Sierka, E.; Baczeńska-Dąbrowska, A.H.; Mojski, J.; Pawluśkiewicz, B.; Sieczko, L.; Kalaji, H.M. Photosynthetic efficiency of perennial ryegrass (*Lolium perenne* L.) seedlings in response to Ni and Cd stress. *Sci. Rep.* **2023**, *13*, 5357. [\[CrossRef\]](#)
21. Rapacz, M.; Sasal, M.; Kalaji, H.M.; Kościelniak, J. Is the OJIP test a reliable indicator of winter hardiness and freezing tolerance of common wheat and triticale under variable winter environments? *PLoS ONE* **2015**, *10*, e0134820. [\[CrossRef\]](#)
22. Stachurska, J.; Rys, M.; Pociecha, E.; Kalaji, H.M.; Dąbrowski, P.; Oklestkova, J.; Jurczyk, B.; Janeczko, A. Deacclimation-Induced Changes of Photosynthetic Efficiency, Brassinosteroid Homeostasis and BRI1 Expression in Winter Oilseed Rape (*Brassica napus* L.)—Relation to Frost Tolerance. *Int. J. Mol. Sci.* **2022**, *23*, 5224. [\[CrossRef\]](#)
23. Rapacz, M.; Hura, K. The pattern of changes in photosynthetic apparatus in response to cold acclimation and de-acclimation in two contrasting cultivars of oilseed rape. *Photosynthetica* **2002**, *40*, 63–69. [\[CrossRef\]](#)
24. Sieczko, L.; Dąbrowski, P.; Kowalczyk, K.; Gajc-Wolska, J.; Borucki, W.; Janaszek-Mańkowska, M.; Kowalczyk, W.; Farci, D.; Kalaji, H.M. Early detection of phosphorus deficiency stress in cucumber at the cellular level using chlorophyll fluorescence signals. *J. Water Land Dev. Spec. Issue* **2022**, 176–186. [\[CrossRef\]](#)
25. Borawska-Jarmułowicz, B.; Mastalerczuk, G.; Dąbrowski, P.; Tuchowska, Ż.; Kalaji, H. Influence of induced drought on photosynthetic performance in *Dactylis glomerata* varieties during the early growth stage. *J. Water Land Dev.* **2024**, *60*, 194–208. [\[CrossRef\]](#)
26. Peña-Olmos, J.E.; Casierro-Posada, F. Chlorophyll Fluorescence in Partially Defoliated Grape Plants (*Vitis vinifera* L. cv. Chardonnay). *Rev. Fac. Nac. De Agron. Medellín* **2013**, *66*, 6881–6889.
27. Mishra, K.B.; Mishra, A.; Novotná, K.; Rapantová, B.; Hodaňová, P.; Urban, O.; Klem, K. Chlorophyll a fluorescence, under half of the adaptive growth-irradiance, for high-throughput sensing of leaf-water deficit in *Arabidopsis thaliana* accessions. *Plant Methods* **2016**, *12*, 46. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Stirbet, A.; Govindjee. The slow phase of chlorophyll a fluorescence induction in silico: Origin of the S-M fluorescence rise. *Photosynth. Res.* **2016**, *130*, 193–213. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Bernát, G.; Steinbach, G.; Kaňa, R.; Govindjee; Misra, A.N.; Prašil, O. On the origin of the slow M–T chlorophyll a fluorescence decline in cyanobacteria: Interplay of short-term light-responses. *Photosynth. Res.* **2018**, *136*, 183–198. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Rapacz, M.; Gasior, D.; Zwierzykowski, Z.; Lesniewska-Bocianowska, A.; Humphreys, M.W.; Gay, A.P. Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of *Festuca pratensis* × *Lolium multiflorum* cultivars. *New Phytol.* **2004**, *162*, 105–114. [\[CrossRef\]](#)
31. Kasajima, I.; Takahara, K.; Kawai-Yamada, M.; Uchimiya, H. Estimation of the relative sizes of rate constants for chlorophyll de-excitation processes through comparison of inverse fluorescence intensities. *Plant Cell Physiol.* **2009**, *50*, 1600–1616. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Brestic, M.; Zivcak, M.; Olsovska, K.; Shao, H.-B.; Kalaji, H.M.; Allakhverdiev, S.I. Reduced glutamine synthetase activity plays a role in control of photosynthetic responses to high light in barley leaves. *Plant Physiol. Biochem* **2014**, *81*, 74–83. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Roháček, K.; Soukupová, J.; Barták, M. Chlorophyll fluorescence: A wonderful tool to study plant physiology and plant stress. In *Plant Cell Compartments—Selected Topics*; Benoît, S., Ed.; Research Signpost: Trivandrum, Kerala, India, 2008; pp. 41–104. ISBN 978-81-308-0104-9.
34. Kalaji, H.M.; Oukarroum, A.; Alexandrov, V.; Kouzmanova, M.; Brestic, M.; Zivcak, M.; Samborska, I.A.; Cetner, M.D.; Allakhverdiev, S.I.; Goltsev, V. Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll a fluorescence measurements. *Plant Physiol. Biochem.* **2014**, *81*, 16–25. [\[CrossRef\]](#)

35. Kalaji, H.M.; Schansker, G.; Ladle, R.J.; Goltsev, V.; Bosa, K.; Allakhverdiev, S.I.; Brestic, M.; Bussotti, F.; Calatayud, A.; Dąbrowski, P.; et al. Frequently asked questions about in vivo chlorophyll fluorescence: Practical issues. *Photosynth. Res.* **2014**, *122*, 121–158. [\[CrossRef\]](#)
36. Tsimilli-Michael, M. Revisiting JIP-test: An educative review on concepts, assumptions, approximations, definitions and terminology. *Photosynthetica* **2020**, *58*, 275–292. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.