AgroLife Scientific Journal - Volume 13, Number 2, 2024
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

POTENTIAL OF THE PROLINE-FLUORESCENT METHOD FOR ASSESSING PLANT DROUGHT TOLERANCE ON THE TEST OBJECT OF OILSEED RADISH

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Abstract

The results of the application of the chlorophyll fluorescence and proline content (PC) methods for assessing the drought tolerance of oilseed radish varieties are presented. The interval of Relative humidity of soil (RHS) 60.8%-35.6% with raising air temperature (0.8°C day^{-1}) and decrease leaf RWC (6.7% day^{-1}) was investigated. An increase F_0 and F_{st} of 1.11 and 0.42 relative fluorescence units (RFU) per 1% decrease in RHS, 2.33 and 0.88 RFU per 1°C increase in air temperature, and 0.35 and 0.13 RFU per 1% decrease in leaf RWC was showed. A decrease F_{pl}, F_m at 6.57 and 50.1 RFU per 1% decrease RHS, 13.8 and 105.2 RFU per 1°C increase temperature and 2.1 and 15.7 RFU per 1% decrease leaf RWC was determined. An increase PC of 0.20 and 0.07 nmol g_{DW}^{-1} per RFU increase F_0 and F_{st} and 1.14 and 8.73 nmol g_{DW}^{-1} per RFU decrease F_{pl} and F_m was noted. An increase PC of 5.74 nmol g_{DW}^{-1} per 1% decrease in RHS and 12.05 nmol g_{DW}^{-1} per 1°C increase in air temperature was proved.

Key words: *stress resistance, stress response, chlorophyll fluorescence, proline test, relative water content.*

INTRODUCTION

Increasing variability of amplitude fluctuations in the hydrothermal regime of the growing season due to climate change remains an important issue for the world to develop adaptive technological strategies to ensure the sustainability of agricultural production (Habib-ur-Rahman et al., 2022; Mazur et al., 2023a).

On the other hand, the genotypic component of species' adaptations to realize the soil and climatic potential of the territories is an important factor in the formation of the state's food security (Kaletnik & Lutkovska, 2021; Berezyuk et al., 2021).

Among the noted features, drought tolerance is the factor that determines the possibility of successful adaptation of a species to the effects of global warming in the format of aridization of the growing season and is desirable for all species grown in the zone of unstable moisture and risky crop production (Mazur et al., 2023). Thermal tolerance guarantees the harmonization of plant growth processes against the background of intense amplitudes of day and night temperatures, which has become even more pronounced in recent years in Europe (Delphine et al., 2023).

Oilseed radish (*Raphanus sativus* L. var. *oleiformis* Pers.) is a cruciferous crop that has become widely used in various systems of organic and conservation agriculture, biofumigation and phytoremediation of soils (Kaletnik et al., 2021; Tsytsiura, 2024). Representatives of the radish genus are widely used in the study of the plant organism's response to stresses of different nature and intensity (Moura et al., 2024). Plants of this genus are considered as effective model biological objects for assessing various types of plant stress resistance (Herppich & Landahl, 2022).

There are different methodological approaches to assessing drought tolerance in crops. They can be divided into a number of systematic areas, such as: direct visual assessment by the state of plants in different soil and climatic zones; induction-stress methods by creating artificial aridity conditions of different durations; indicative assessment methods based on proven test signs for assessing the stress response of plants at the level of photosynthetic system tissues; assessment of the biochemical response of plants to stressful stimuli (Seleiman et al., 2021). The problematic aspect is the selection of the optimal option from the spectrum of these methods, which would provide a reliable assessment of plants for drought and heat resistance and would be effective in terms of application (Osmolovskaya et al., 2018; Takahashi et al., 2020).

The tissue organoleptic system for identifying drought tolerance in the vast majority of cases is of a statement nature for a certain phenological phase of crop development or in the case of studying certain reactions to possible range changes in the stress factor of high temperatures or moisture deficit. This is not enough to adequately assess the response of the plant organism in view of the change in physiological and adaptive resistance of plants in the process of their stage development and biological aging, especially under a favorable or unfavorable set of additive factors and edaphic conditions (Movahedi et al., 2023). It is noted that tissue laboratory methods for assessing drought tolerance, in the presence of pre-adaptive plant responses and a full-fledged biochemical physiological signaling system, should be combined with the search for biochemical and photochemical mechanisms of plant stress response that will form a reliable dual system of phenotypic and biochemical character (Wang et al., 2024).

In terms of biochemical evaluation, the release of physiologically active substances as a plant organism's response to stress was studied. These substances include proline, sucrose, polyols, trehalose and quaternary ammonium compounds such as glycine betaine, alinine betaine, proline betaine and pipecolate betaine. Among these compounds, proline was identified in the case of stress response to water deficit, salinity, low temperature, heavy metal exposure UV radiation, etc. (Hayat et al., 2012; Raza et al., 2023).

Differences in the use of the proline test for assessing drought tolerance of different varieties of the same species were noted. This requires an additional assessment of changes in proline concentration against the background of different variants of moisture deficit in view of the phenological development of plants to identify critical periods of sensitivity to soil drought (Per et al., 2017; Alvarez et al., 2021).

The phenomenon of chlorophyll fluorescence induction has been studied in recent years as a photochemical indication of drought resistance (Zhuang et al., 2020). The change in the nature of the Kautsky curve (inductive curve) under the influence of stress factors of different nature was determined (Plich et al., 2020; Hu et al., 2023). It should be noted that modern approaches to application of individual parameters of the chlorophyll fluorescence induction curve are different. This requires a certain generalization for different types of plants in order to apply in scientific practice the identification of its stress state (Barboričová et al., 2022; Makhtoum et al., 2023).

Based on the above statements, the aim of the study was to investigate the effectiveness of identifying the stress response of oilseed radish plants both in terms of changes in the dynamics of the chlorophyll fluorescence induction curve and changes in the concentration of proline in assimilative tissues under simulated drought conditions. This allowed to deepen knowledge in the field of formation of adaptive stress responses of plants to abrupt changes in abiotic environmental factors and further application of certain mechanisms in the formation of adaptive elements of cruciferous crops cultivation technology in general.

MATERIALS AND METHODS

The experiment was was carried out during 2024 at Vinnytsia National Agrarian University. (N 49°12′34″, E 28°24′21″) on two oilseed radish varieties of remote breeding and geographical origin 'Zhuravka' and 'Alfa'. According to the results of long-term zonal environmental testing, 'Zhuravka' was classified as a genotype with an average level of drought tolerance and 'Alfa' with a high level.

Plants were grown in laboratory controlled conditions using specialized containers for growing (Figure 1). To maximize the adaptation of the cultivation, the soil substrate selected from the experimental field with a thickness of 0-30 cm was used. The mechanical composition of the soil substrate was sand 12.03-14.32%, silt 55.86-57.79%, clay 29.35-30.21%. The agrochemical potential of the soil (type of soil Luvic Greyic Phaeozem) was as follows: humus content 2.72%, easily hydrolyzed nitrogen 82 mg kg^{-1} of the soil, mobile phosphorus 157 mg kg^{-1} of the soil, exchangeable potassium 102 mg kg^{-1} of the soil, pH_{KCl} 5.8. Each container for growing plants contained 2 kg of soil substrate, which was moistened to 75% of the field moisture capacity by direct irrigation with distilled water.

The seeds were sown in soil-filled containers with a distance of 2 cm between them to a depth of 2 cm after preliminary soaking and disinfection in a weak solution (0.2%) of potassium permanganate.

The blocks for growing in each variant of the experiment were laid out in quadruplicate. Plants were grown under controlled conditions at a temperature of +20/+15°C (day/night), light intensity of 720 μmol $(m^2\ s)^{-1}$, photoperiod of 16/8 h (day/night), relative humidity of 75 ± 5%, and substrate moisture content was maintained at 60-65% of the full moisture capacity.

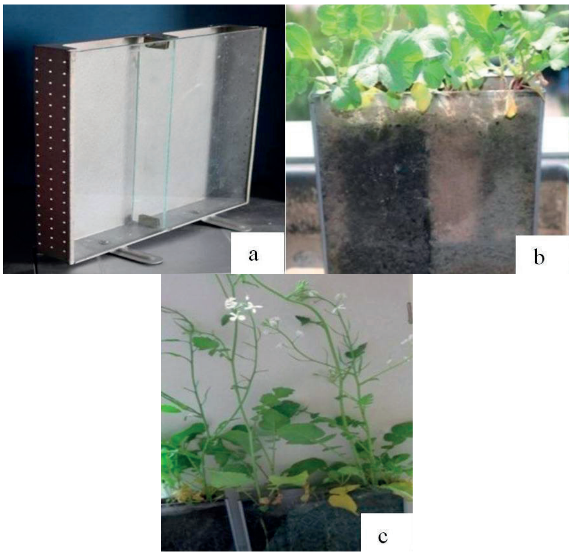


Figure 1. System of specialized containers for growing oilseed radish plants, 2023 (a - general view of the containers; b - oilseed radish plants at the rosette phase (BBCH 17-19) and flowering phase (BBCH 63-65)

The moisture level was monitored using an AMTAST S-1 probe sensor. Full spectrum phytolamps APL 100 W with a time timer were used to illuminate the plants. Plants were grown on an unfertilized background.

Drought simulation was carried out by stopping irrigation of the corresponding experimental variants for 15 days from the level of Relative humidity of soil (RHS) from RHS 60.8% (no drought) to the level of reaching 35.6% (30%< RHS ≤ 40% (severe drought) according to the classification standard of drought grade (according to Yan et al, 2023) with a dynamic increase of the growing temperature with a gradient of 0.8°C day^{-1} for a comprehensive simulation of a combined drought (beginning of the period was 20°C - end of the period was 32°C) (according to Osmolovskaya et al., 2017). The control was a variant with normal moisture The simulation was applied to plants that were at the beginning of the respective phenological phases (rosette (BBCH 17–19), stemming (BBCH 35-37) and flowering (BBCH 63-65) (according to UPOV, 2017) for both varieties), taking into account the intensity of growth processes and sensitivity to abiotic environmental factors of this plant species (Tsytsiura, 2020). This ensured that the main stage of stress coincided with the desired daily periodization of phenophases. The chlorophyll fluorescence was measured on day 7 in the experimental variants.

The proline content (PC, %) in leaf tissues determined in the interpretation of Zegaoui et al. (2017). For homogenization of the leaves, 0.5 g weight was used with the addition of 10 ml of cooled 3% sulfosalicylic acid. The resulting homogenate was cinefuged for 15 minutes. After that, 2 milliliters of the solution was filtered and taken. To this solution was added 2 milliliters of acidic ninhydrin and the same volume of ice-cold acetic acid. The mixture was incubated for 1 h at 100°C. After that, the solution was cooled and 4 ml of toluene was added. Spectrometric analysis of the solution was performed at 520 nm (Spectrophotometer 722G, Shanghai Drawell Scientific Instrument Co., Ltd., China) with a control toluene solution. For the preparation of the calibration solution, 25 mg of L-proline (BioVit, Ukraine) was used, which was diluted to 250 ml to prepare standard solutions of 1, 2, 3, 4, 5 and 6 mg ml^{-1}. The proline content was determined by comparing the standard curve of the corresponding standard concentrations. The proline content was expressed as nmol g_{DW}^{-1} (at a standard molecular weight of 115.0633 g mol^{-1}).

The relative water content of leaves (RWC) was determined according to Zegaoui et al. (2017) (Equation 1). For this purpose, the fresh weight (FW) in the leaf sample was determined. Leaf excisions were kept at 4°C for 24 hours. After that, the weight of the leaves at full turgor (TW) was determined. The procedure was completed by drying the leaves in an oven at 70°C to obtain the dry weight of the leaves (DW).

$$RWC(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100 \qquad (1)$$

where: FW - fresh weight of the leaves (g), TW - turgor weight of the leaves (g), DW - dry weight of the leaves (g).

To determine the parameters of chlorophyll fluorescence induction, a portable single-beam fluorometer 'Floratest' was used (Posudin et al., 2010). The device had an optoelectronic sensor (irradiation wave parameter 470 ± 15 nm, irradiation area 15 mm^2 at an illumination at the irradiation point of 2.4 W m^{-2}) (Figure 2).

The fluorescence measurement range was 670-800 nm with a second-by-second (0-90 seconds interval) recording of the indicators. The measurements were made on vegetative plants after the leaves had adapted to the darkness for 10 minutes. The sample consisted of 28 plants in 4 replicates (*N = 112* measurements). The measurements were made in relative fluorescence units (RFU). The system of accounting and curve construction was based on the Kautsky' effect curves (Figure 3) with the basic curve indicators according to the standard protocol for determining the induction of chlorophyll fluorescence (Brestic & Zivcak, 2013; Kalaji et al., 2017) and the indicators determined on its basis (Table 1, (Equations 2-16).

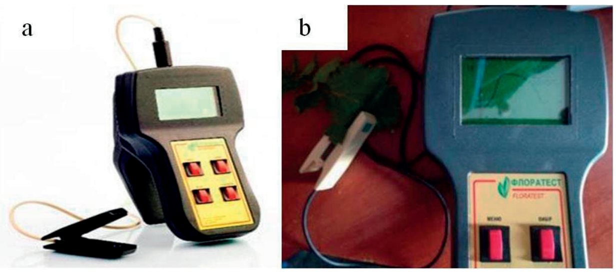


Figure 2. General view of the portable fluorometer 'Floratest' (a) and the device during measurements (b)

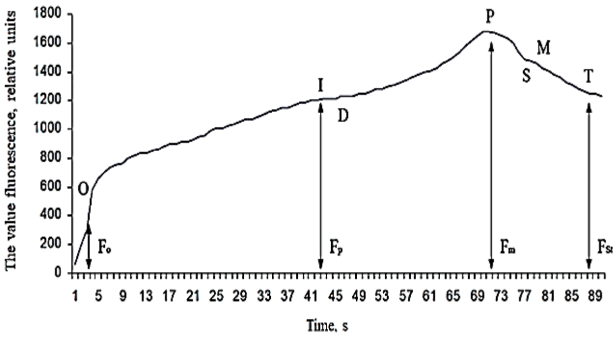


Figure 3. Model typical plot of the chlorophyll fluorescence induction curve (according to Kalaji et al., 2017) (F_0 – initial fluorescence; F_p (or F_{pl}) – fluorescence of the 'plateau' zone; F_m – maximum fluorescence; F_{st} – zone of steady state fluorescence)

Table 1. Derivative indicators of the CFI curve (formed by Brestic & Zivcak, 2013; Tsytsiura, 2022)

The CFI curve indexes	The applied formula
Fluorescence rise (dF_{pl})	$dF_{pl} = F_{pl} - F_0$ (2)
Maximum variable fluorescence (F_v)	$F_v = F_m - F_0$ (3)
Index of the effect of exogenous and endogenous factors	dF_{pl} / F_v (4)
Photochemical efficiency or quantum efficiency (EP)	$EP = F_v / F_m$ (5)
Photochemical quenching (Q_{ue})	$Q_{ue} = F_0 / F_v$ (6)
Leaf water potential (L_{wp})	$L_{wp} = F_m / F_0$ (7)
Plant viability index (RF_d)	$RF_d = (F_m - F_{st}) / F_{st}$ (8)
Indicator of endogenous (stress) factors (K_{ef})	$K_{ef} = F_{st} / F_m$ (9)
Value of photochemical quenching of fluorescence (QP)	$QP = (F_m - F_{st})/(F_m - F_0)$ (10)
Index of the efficiency of the primary reactions of photosynthesis (K_{prp})	$K_{prp} = F_v / F_0$ (11)
Fluorescence decay coefficient (K_{fd})	$K_{fd} = F_m / F_{st}$ (12)
Relative change of fluorescence at time t (V_t)	$V_t = (F_{st} - F_0)/(F_m - F_0)$ (13)
Normalized area for rising parts of the curve (NA_{rp})	$NA_{rp} = \frac{A}{(F_m - F_0)}$ (14)
Normalized area for declining parts of the curve (NA_{dp})	$NA_{dp} = \frac{A}{(F_m - F_{st})}$ (15)
Dynamics symmetry ratio (DSR)	$DSR = NA_{rp}/NA_{dp}$ (16)

where: F_0 – initial fluorescence, F_{pl} – fluorescence of the 'plateau' zone, F_m – maximum fluorescence, F_{st} – steady state fluorescence, A – area above the CFI curve, (RFU s) (calculated using the trapezoidal integration method - Rohde et al., 2012)

On another laboratory block of containers with plants under the same growing conditions, records were made in conjugate dynamics with changes in the RWC of oilseed radish leaves of the studied varieties during the flowering phase (BBCH 61-67) for 15 days after the termination of moistening of containers with plants every two days. After recording the chlorophyll fluorescence values for the same plants, the relative water content (RWC) in the leaves and proline content were determined using the above methods.

The results were subjected to statistical analysis using standardized indices according to Sneyd et al. (2022) using Statistica 10 (Dell Software Company, USA, 2013) and Past 4.13 (Hammer, Norway).

RESULTS AND DISCUSSIONS

It was determined that the character of the CFI curve had significant differences within the phenological phases under both normal and stressful conditions. Under normal conditions, a steady increase in the dynamics of the curve (for the peak value and the intensity of its achievement) from the rosette phase to the flowering phase was noted for both varieties.

The maximum of F_m was reached in the flowering phase (Figure 4).

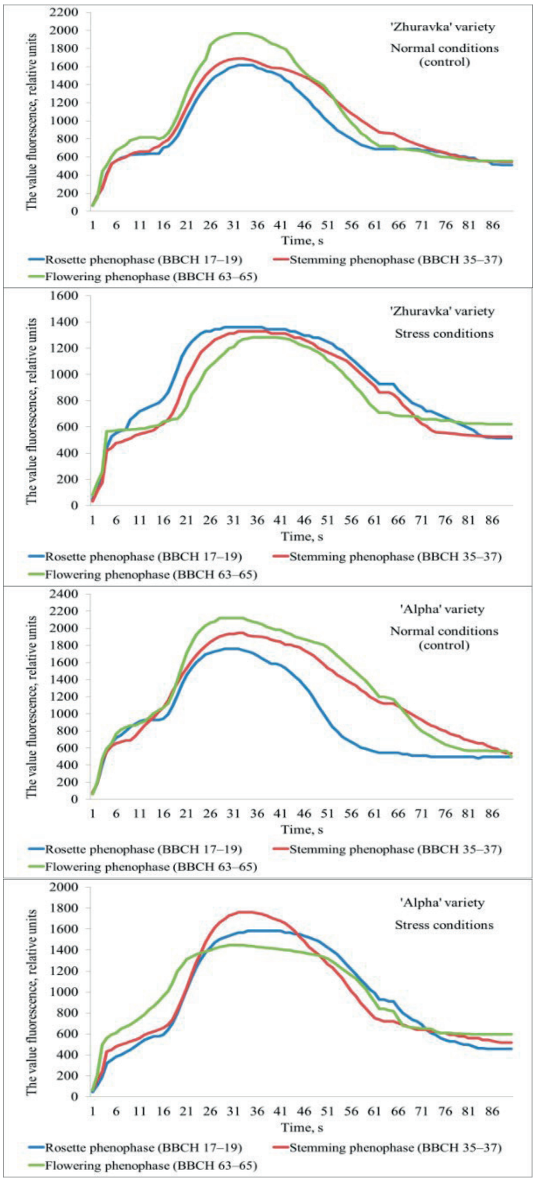


Figure 4. CFI curves of oilseed radish varieties under different conditions for various phenological stages

The nature of the growth coefficients of the ratios of the flowering phase to other accounting phases was also in favor of the flowering phase. For the variety 'Zhuravka', the average value of the baseline CFI curve was 1.16 for the rosette phase and 13 for the stemming phase For the variety 'Alpha', these indicators were at the level of 1.05 and 1.06. For the variety 'Alpha', in addition, a decrease in F_0 and F_{st} indicators by 2.3 and 5.9%, respectively, was noted in comparison of flowering and stemming phenophases. This ledto a change in the ordinal height of the fluorescence curve points at different phenological stages of the varieties. It was confirmed by the values of NA_{rp}, NA_{dp} and SDR (Table 2).

Table 2. Baseline and calculated values of the CFI curve in oilseed radish varieties at different phenological phases and drought degree (relative units of fluorescence)

Experimental variant			Basic indicators			
			F_0	F_{pl}	F_m	F_{st}
'Zhuravka' variety						
I	90.7	1**	417 ± 53^{a}	638 ± 41^{c}	$1,617 \pm 150^{c}$	512 ± 63^{a}
I	89.4	2	405 ± 74^{a}	659 ± 99^{d}	$1,689 \pm 203^{d}$	547 ± 90^{c}
I	92.1	3	459 ± 59^{b}	816 ± 119^{e}	$1,968 \pm 243^{e}$	555 ± 73^{c}
II	52.2	1	444 ± 51^{b}	572 ± 60^{b}	$1,360 \pm 190^{a}$	534 ± 72^{a}
II	51.9	2	457 ± 65^{c}	512 ± 68^{a}	$1,328 \pm 237^{a}$	556 ± 71^{b}
II	51.5	3	566 ± 73^{d}	588 ± 101^{b}	$1,282 \pm 252^{b}$	621 ± 81^{d}
			dF_{pl}	F_v	dF_{pl}/F_v	EP
I	90.7	1*	221	1200	0.184	0.742
I	89.4	2	254	1284	0.198	0.760
I	92.1	3	357	1509	0.237	0.767
II	52.2	1	128	916	0.140	0.674
II	51.9	2	55	871	0.063	0.656
II	51.5	3	22	716	0.031	0.559
			L_{wp}	Q_{ue}	RF_d	K_{ef}
I	90.7	1	3.88	0.35	2.158	0.317
I	89.4	2	4.17	0.32	2.088	0.324
I	92.1	3	4.29	0.30	2.546	0.282
II	52.2	1	3.06	0.48	1.547	0.393
II	51.9	2	2.91	0.52	1.388	0.419
II	51.5	3	2.27	0.79	1.064	0.484

			QP	K_{prp}	K_{fd}	V_t	NA_{rp}	NA_{dp}	SDR
I	90.7	1	0.921	2.878	3.158	0.083	18.7	36.92	0.51
I	89.4	2	0.889	3.170	3.088	0.111	17.7	33.24	0.53
I	92.1	3	0.936	3.288	3.546	0.064	17.4	37.66	0.46
II	52.2	1	0.902	2.063	2.547	0.098	15.3	26.45	0.58
II	51.9	2	0.886	1.906	2.388	0.114	20.2	29.57	0.68
II	51.5	3	0.923	1.265	2.064	0.077	19.5	26.52	0.73

'Alpha' variety						
I	90.7	1	404 ± 46^{a}	928 ± 73^{f}	$1,760 \pm 129^{b}$	496 ± 55^{b}
I	89.4	2	454 ± 52^{c}	688 ± 55^{d}	$1,944 \pm 247^{d}$	540 ± 86^{d}
I	92.1	3	472 ± 61^{b}	764 ± 85^{e}	$2,118 \pm 291^{e}$	565 ± 82^{c}
II	52.2	1	426 ± 59^{b}	544 ± 50^{a}	$1,584 \pm 105^{a}$	510 ± 60^{a}
II	51.9	2	480 ± 62^{d}	592 ± 71^{b}	$1,760 \pm 116^{b}$	582 ± 97^{c}
II	51.5	3	496 ± 87^{e}	608 ± 83^{c}	$1,447 \pm 199^{c}$	597 ± 94^{e}
			dF_{pl}	F_v	dF_{pl}/F_v	EP
I	90.7	1	524	1356	0.386	0.770
I	89.4	2	249	1505	0.165	0.774
I	92.1	3	292	1646	0.177	0.777
II	52.2	1	118	1158	0.102	0.731
II	51.9	2	112	1280	0.088	0.727
II	51.5	3	112	951	0.118	0.657
			L_{wp}	Q_{ue}	RF_d	K_{ef}
I	90.7	1	4.36	0.30	2.548	0.282
I	89.4	2	4.43	0.29	2.600	0.278
I	92.1	3	4.49	0.29	2.749	0.267
II	52.2	1	3.72	0.37	2.106	0.322
II	51.9	2	3.67	0.38	2.024	0.331
II	51.5	3	2.92	0.52	1.424	0.413

			QP	K_{prp}	K_{fd}	V_t	NA_{rp}	NA_{dp}	SDR
I	90.7	1	0.932	3.356	3.548	0.068	14.2	41.5	0.34
I	89.4	2	0.933	3.428	3.600	0.067	15.5	29.01	0.53
I	92.1	3	0.943	3.487	3.749	0.057	15.1	29.84	0.51
II	52.2	1	0.927	2.718	3.106	0.073	20.5	28.43	0.72
II	51.9	2	0.920	2.667	3.024	0.080	20.8	35.14	0.59
II	51.5	3	0.894	1.917	2.424	0.106	10.8	21.04	0.51

Note: I – normal conditions; II – stressed conditions; * – RWC (%, leaves); **1 – rosette phenophase (BBCH 17-19), 2 – stemming phenophase (BBCH 35-37), 3 – flowering phenophase (BBCH 63-65). Different letters indicate values that differed significantly based on the results of the comparison using the Tukey's test with the Bonferroni correction.

For the 'Zhuravka' variety, averaged over the phenological phases, these indicators were higher by 20.4%, 9.9% and 13.4%, respectively. Varietal specificity of CFI curve profiles was also determined (Table 2). On average, 'Zhuravka' variety had lower values of F_0 (-2.6% compared to 'Alpha' variety), F_{pl} (-11.2%) and F_m (-9.4%) and higher values of F_{st} (+0.8%).

The maximum values of the main derivative indices, with the exception of V_t, Kef and Que, were observed in the flowering phase. For 'Zhuravka' variety in comparison to the rosette and stemming phases, this increase was 17% and 13.8%. For the 'Alpha' variety, these figures were 8.4% and 8.0%.

For the same indicators, the reverse nature of formation was noted with an average decrease for 'Zhuravka' variety of 14.3% (compared to the rosette phase) and 19.7% (to the stemming phase). For the 'Alpha' variety, these indicators were at the level of 8.6% and 7.2%, respectively. The obtained values, taking into account a number of studies (Zhu et al., 2021; Barboričová et al., 2022; Xue et al., 2022), with a downward trend, was proved the increase in the stress sensitivity of oilseed radish plants with its consistent phenological development. At the same time, the depressive effect of a decrease in soil moisture will be predicted to be more noticeable with a simultaneous increase in air temperature.

This is consistent with the results of Kalaji et al. (2017), according to which the nature of the amplitude fluctuations of the chlorophyll fluorescence curve was an identifier of stress responses in different plant species. Stress-sensitive genotypes was characterized by both intensive growth in the F_0-F_m section of the curve and intensive decline in the F_m-F_{st} section (Brestic and Zivcak, 2013). For the studied varieties SDR was 0.44 wich allowed to classify the nature of CFI curves as asymmetric with significantly higher growth rates on the curve segment F_0-F_m compared with the F_m-F_{st} segment.

It has also been established that the pronounced asymmetry with the dominance of the increasing part of the CFI curve was characteristic of plant species with increasing resistance to stress upon reaching the critical period of photoassimilation (Guidi et al., 2019; Pérez-Bueno et al., 2019). This is also confirmed by the obtained value of the indicator of leaf water potential (Lwp), which was 13.3-15.8% higher during the flowering phase and which (according to Zhao et al., 2020; Xing et al., 2022) was a criterion for the total water content of leaf tissues and its overall need for sufficient moisture at physiologically optimal levels of transpiration.

The identification of the flowering phase as critical from the point of view of drought resistance for both varieties of oil radish is confirmed by the maximum value of the index of plant viability (RF_d) against the background of the minimum value of the index of endogenous (stress) factors (K_{ef}). Based on data from studies by Chen et al. (2019) and Rao et al. (2021) obtained data confirmed the dependence of the physiological activity of the photosystem of plants on their sequential phenological development. Based on this, it has been proven that CFI curves can be used to identify the phenological development of plants. This was consistent with the fact that the photosynthetic activity of plants has a high positive correlation with the achievement of critical periods of its development (Kalaji et al., 2016; Jonathan, 2017). For most species (cruciferous in particular), this period covered the phenological phases from budding to flowering (El Idrissi et al., 2023).

These generalizations was confirmed by the data of the CFI curve under severe stress (Table 2, Figure 4). Such reaction was observed both when comparing data for different phenological phases and when comparing studied varieties.

For varieties, a different pattern of changes in the basic indicators of the CFI curve was found in the comparison of the same phenological phases of accounting. For the 'Zhuravka' variety, in the rosette phase, in comparison with stressful-normal growth conditions, an increase in F_0 and F_{st} by 6.5% and 4.3% was noted, while F_{pl} and F_m decreased by 10.3% and 15.9%. For the stemming phase, the same indicators had following dynamics: +12.8%, +1.6%, -22.3% and -21.4%. For the flowering phase, similar indicators were +23.3%, +11.9%, -27.9% and -34.9%. For the variety 'Alpha', with the similarity of the formation of the directions of dynamics of changes in the basic indicators of the CFI curve, the magnitude of the changes themselves was significantly lower. Thus, for the phenological phases of rosette and stemming, the intensity of changes was on average 6.3% lower. For the flowering phase, the increase in F_0 and F_{st} in comparison with normal conditions was lower than in 'Zhuravka' by 17.3% and 5.9%. The value of the decrease in F_{pl} and F_m was also 10.4% and 4.9% lower for the same varietal comparison. The maximum reductive changes in chlorophyll fluorescence indices were found in the flowering phase for both oilseed radish varieties. This confirmed the conclusions about the difference in the stress response of plants at different stages of development. The influence of drought led to a change in the coordinate points of the CFI curve. Gradually from the rosette phase to the flowering phase under the same stress factors, a decrease in the ordinal position of the curve arm in the F_0-F_{pl} area and the total amplitude of the F_{pl}-F_m growth was observed. Stress conditions led to a decrease in the angle of inclination of the CFI curve relative to the abscissa axis in the F_m-F_{st} section. As a consequence of such dynamism of the curve, fluorescence changes in time (V_t) increased under stress conditions for all accounting phenophases with a maximum value for the flowering phenophase. The identified changes in the nature of the CFI curve affected the values of NA_{rp}, NA_{dp} and SDR. The overall decrease in the amplitude of the curve led to an adequate reduction in asymmetry and an increase in the normalized area above the curve by 8.5-12.8% in the flowering phase for both varieties.

Other indicators of the CFI curve had a changed pattern of formation. Steady decline in the water potential of plant leaves (L_{wp}) under stress was determined with a minimum value at the flowering stage. In particular, during the flowering phase, compared to normal conditions, its total decrease was 47.2% in 'Zhuravka' variety and 35% in 'Alpha' variety. During the same phase, the plant viability index (RF_d) under stress conditions also had a steady downward trend from the rosette phase to the flowering phase with a minimum value at the flowering phase. In comparison with normal conditions for the same phenophase, the decrease was 58.2% in 'Zhuravka' and 48.2% in 'Alpha' variety. These patterns was positively correlated with such indicators as the indicator of endogenous (stress) factors (K_{ef}) and fluorescence decay coefficient (K_{fd}), given the steady increase of the former (1.7 times in 'Zhuravka' and 1.5 times in 'Alpha' variety) and the steady decrease of the latter (-41.8% and -35.3% for the same varieties) under a number of stressful conditions from the rosette phase to the flowering phase. As a result, due to a gradual decrease in the indicators that determine the dynamic performance of phytochemical activity (F_v, EP and K_{prp}) (according to Kalaji et al., 2016), the effect of stress quenching of chlorophyll fluorescence induction was established with its maximum expression in the flowering phase. The above-mentioned indicators reduction gave grounds to confirm the status of 'Alpha' variety as more drought-resistant according to the results of the zonal environmental test. This confirmed the possibility of using the chlorophyll fluorescence method to evaluate cruciferous varieties for drought tolerance. This methodological approach has also been successfully used in terms of breeding and genetic analysis according to the full Jinks-Heyman scheme (Tsytsiura, 2022; 2023).

The results obtained in these studies are positively consistent with the conclusions drawn in other studies. Thus, an increase in F_0 under stress conditions indicated a less efficient transfer of excitation energy between pigment molecules in the light-harvesting antenna of plant photosystem II (PSII) when thylakoids are damaged and the system itself is inactivated (Kalaji et al. 2017; Ashrostaghi et al., 2022).

The stressful state of the plants was also confirmed by fixing the F_{pl} index on the segment of dynamic growth of the CFI curve. With low stress resistance of plants to the corresponding stress factor, the plateau zone was well expressed and fixed in a long dimension, forming a well-resistant linear area on the graph in the interval between F_0 and F_m (Brestic & Zivcak, 2013). It is also known (Brestic & Zivcak; Herritt et al., 2020) that a decrease in F_m indicated a stressful state of the photosystem and limited recovery of photosystem II (PSII) electron acceptors. The influence of stresses (high and low temperatures, moisture deficit) on the value of F_{st} was also noted. It has been established

(Kalaji et al., 2017; Persˇic´ et al., 2022) that under intense stress and natural stage senescence of leaves, the value of F_{st} increased and the time of reaching it decreased, providing an increase in the relative change of fluorescence at time t (V_t). Similar reactions were observed in these studies. However, certain differences were also found. In particular, the nature of fixation of F_{pl} in oilseed radish under stress conditions occurs on a fuzzy increasing part of the curve, in contrast to normal growth conditions. This was typical for plants with certain pre-adaptive responses to stress in the format of photochemical functional restructuring of the photosynthetic apparatus (Hasanuzzaman, 2020). The duration of fixation of the same values in drought conditions for the flowering phase for the F_{st} indicator in the 'Zhuravka' variety was up to 7 s, and for the 'Alfa' variety - up to 5 s. Under these conditions, minimal ordinate changes of 2-11 relative fluorescence units were also noted for this indicator. Such results contradict the statement of Ač et al. (2015) that temperature and humidity cause F_{st} to decrease. It was noted that the value of the EP index against the background of dark adaptation of plants characterized the quantum potential of photosystem II. Under normal conditions, its value for most plant species was 0.830 (Brestic & Zivcak, 2013; Guidi et al., 2019). It was determined that a decrease in ER was a sign of stress (Feria-Gómez et al., 2022). Under normal conditions of moisture and temperature, the value of this indicator in oilseed radish varieties was in the range of 0.742-0.777. In view of this, the value of 0.830, characteristic of most plant species, should be considered achievable under ideal conditions of growth and development.

On the other hand, it maked sense to apply the category of pre-stress condition or selection of growing conditions for the control variant to achieve the specified ER value. Attention should also be focused on the variability of the point fixation of the CFI curve within the plant, which is rarely used in the system of stress resistance analysis by chlorophyll fluorescence. According to Schreiber & Klughammer (2021), assessing the degree of variation of the CFI curve points will allow determining the nature of their formation. The results of this assessment for oil radish varieties are shown in Figure 5.

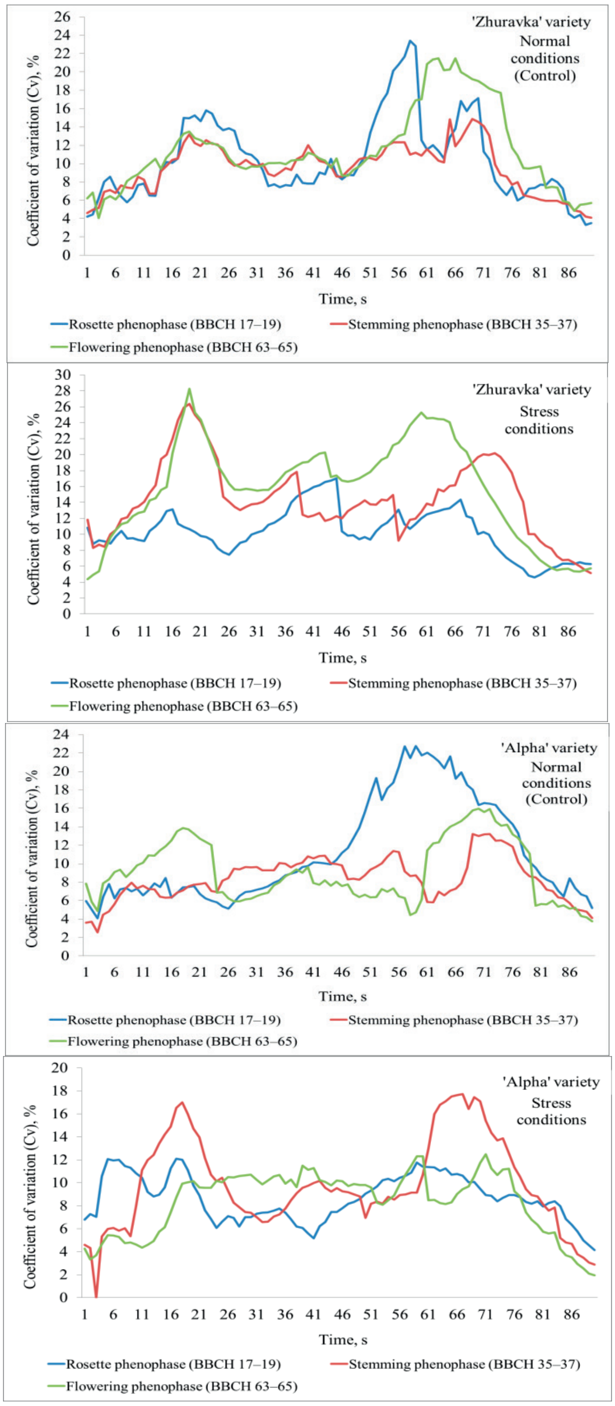


Figure 5. Variation of the CFI curve fixation points under different growing conditions at the corresponding phenological stages in oilseed varieties (for *N=112* at each point of fixation)

The graphical interpretation of the variation showed an increase in the variation of the points of fixation of the CFI curve with an increase in the stress load on the plant. The maximum variation was observed at the fixation points of 16–31 and 51-77 seconds.

This interval corresponded to the areas of the F_{pl}-F_m curve and from the beginning of the intense fluorescence decline to the point of fixation F_{st}. The difference in the formation of peak values of the coefficient of variation depending on the phenological phase of plant development was found.
For 'Zhuravka' variety, under normal conditions, the level of variation at the rosette and flowering stages was on average 3.9% higher than at the stemming stage. Under stressful conditions, the variation at the flowering stage was maximum at the flowering stage with an average growth coefficient of 1.17 to the rosette stage and 1.07 to the stemming stage.
Under stress, a general increase of 1.4-2.0 times was noted, depending on the phenophase of plants for the area of formation of the F_m index on the CFI curve. For the 'Alpha' variety, with the same trends of growth variation under stressful conditions, its value was close to the rosette and flowering phase under normal conditions and was significantly higher in the stemming phase under stressful conditions.
The average growth coefficient was 1.53 for the rosette phase and 1.19 for the flowering phase. These results was confirmed a number of conclusions (Brestic & Zivcak, 2013; Dutta et al., 2017) that the physiological mechanisms of chlorophyll fluorescence had the most complex response mechanisms at the initiation and completion of the process.
Stress created prerequisites for the variability of these mechanisms in the photosystem mechanisms of a given plant species (Larouk et al., 2021), and the plant itself, even within the same phenological phase, had leaves of different ages, which formed differences in chlorophyll fluorescence (Pérez-Bueno et al., 2019).
Based on this, the need to form an accounting database of indicators in at least 4-5 leaves of the middle tier of the plant (or typical morphological development for this phenophase) was determined when generating experimental data. A comparative assessment of the effect of reducing leaf water content in the format of reducing its RWC on the chlorophyll fluorescence of oilseed radish varieties is shown in Figure 6.

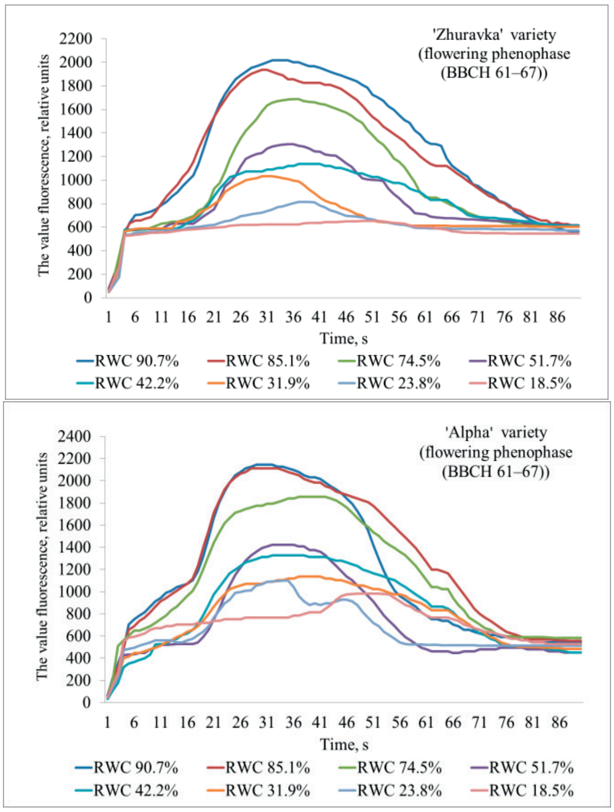


Figure 6. CFI curves of oilseed radish varieties against the background of leaf RWC values for the flowering phase (BBCH 61-67)

This studies have proven a number of practical applications of the chlorophyll fluorescence method for the clear identification of the adaptive potential of plants in terms of their drought tolerance.
The existence of a dynamic positive relationship between the decrease in leaf RWC and the formation of a stressful CFI curve in oilseed radish was established. This is also confirmed by the research of Ievinsh (2023). The decrease in the angle of inclination of the curve on the F_0-F_m segment with a decrease in soil moisture was proved. At the same time, the clarity of fixation of the F_m indicator decreases with the formation of a plateau-like structure with a general decrease in the F_m value. A decrease in the gradient of fluorescence units for the duration in seconds of the F_m-F_{st} segment of the CFI curve was also observed.
Actually, the identification of the plateau zone (F_{pl}) on the curve was extended in the direction of the abscissa axis. As a result, a pronounced and long plateau was formed. The time of fixation of the Fst index is shifted from the time mark of 76-81 seconds to 56-75 seconds.

In the extreme stress state, which according to Kalaji et al. (2016) is determined by the lack of clear differentiation of F_{pl} and F_{st} with a minimum F_m, the graph acquired an almost straight line with a small amplitude at the place of F_m fixation.

This condition indicated functional destruction of chloroplasts (according to Batool et al. (2022)). For 'Zhuravka' variety, this level of stress was achieved at RWC 24.7%, and for 'Alfa' variety 18.5%. This confirmed the conclusions about the drought tolerance of oilseed radish varieties. This character of the CFI curve formation had different symmetry in terms of SDR (Figure 7). For the less drought-resistant 'Zhuravka' variety, a gradual increase in asymmetry was determined with the dominance of NA_{dp}. For the 'Alpha' variety, an increase in asymmetry was observed with the dominance of the NA_{rp} index. At the same time, the asymmetry of the CFI curve in the 'Zhuravka' variety was 0.39. The average value of asymmetry in the 'Alpha' variety was 1.37 with an ideal asymmetry index of 1. Based on this, the expediency of using the SDR criterion in the overall assessment of plant drought tolerance in the array of chlorophyll fluorescence indicators was proved. This was consistent with the findings of Tschiersch et al. (2017) and Stirbet et al. (2018) regarding the need to analyze CFI curves within certain plant genotypes.

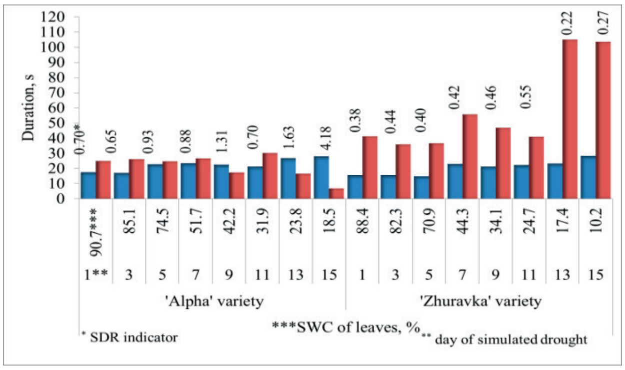


Figure 7. Values of NA_{rp} and NA_{dp} indicators in oilseed radish varieties depending on the SWC of leaves

The dynamic nature of the CFI curve for reducing RWC in the digitized version is presented in the Table 3. According to the LSD indicator for *p<0.05*, a significant difference between the RWC gradations of the leaves was determined. This indicator was 74.5% for the 'Alfa' variety and 82.3% for the 'Zhuravka' variety.

The regularity noted on the curves was found to increase the basic indicators F_0 and F_{st} with a decrease in F_{pl} and F_m for the 'Alpha' variety. In 'Zhuravka' variety, similar changes for the first two indicators were observed up to 9 days of drought simulation.

Table 3. Baseline and calculated values of the CFI curve in oilseed radish varieties for different levels of RWC at the flowering phase (BBCH 61-67) (relative fluorescence units)

DSD*	RWC, %		Estimated and derivative CFI curve indicators							
			'Alpha' variety				'Zhuravka' variety			
	1*	2	F_0	F_{pl}	F_m	F_{st}	F_0	F_{pl}	F_m	F_{st}
1	90.7	88.4	480	752	2,144	574	544	704	2,016	561
3	85.1	82.3	483	688	2,112	572	560	656	1,936	608
5	74.5	70.9	484	649	1,856	582	566	580	1,689	612
7	51.7	44.3	488	611	1,424	586	572	583	1,305	616
9	42.2	34.1	496	602	1,328	592	578	585	1,136	613
11	31.9	24.7	524	587	1,136	598	564	590	1,032	602
13	23.8	17.4	534	583	1,102	604	536	558	814	571
15	18.5	10.2	552	570	984	612	528	555	652	544
LSD_{05}			3.8	5.7	33.7	3.5	3.9	4.6	78.4	3.2
RWC, %			dF_{pl}	F_v	dF_{pl}/F_v	EP	dF_{pl}	F_v	dF_{pl}/F_v	EP
1	90.7	88.4	272	1,664	0.163	0.776	160	1,472	0.109	0.730
3	85.1	82.3	205	1,629	0.126	0.771	96	1,376	0.070	0.711
5	74.5	70.9	165	1,372	0.120	0.739	14	1,123	0.012	0.665
7	51.7	44.3	123	936	0.131	0.657	11	733	0.015	0.562
9	42.2	34.1	106	832	0.127	0.627	7	558	0.013	0.491
11	31.9	24.7	63	612	0.103	0.539	26	468	0.056	0.453
13	23.8	17.4	49	568	0.086	0.515	22	278	0.079	0.342
15	18.5	10.2	18	432	0.042	0.439	27	124	0.218	0.190
RWC, %			L_{wp}	Q_{ue}	RF_d	K_{ef}	L_{wp}	Q_{ue}	RF_d	K_{ef}
1	90.7	88.4	4.47	0.29	2.735	0.268	3.71	0.37	2.594	0.278
3	85.1	82.3	4.37	0.30	2.692	0.271	3.46	0.41	2.184	0.314
5	74.5	70.9	3.83	0.35	2.189	0.314	2.98	0.50	1.760	0.362
7	51.7	44.3	2.92	0.52	1.430	0.412	2.28	0.78	1.119	0.472
9	42.2	34.1	2.68	0.60	1.243	0.446	1.97	1.04	0.853	0.540
11	31.9	24.7	2.17	0.86	0.900	0.526	1.83	1.21	0.714	0.583
13	23.8	17.4	2.06	0.94	0.825	0.548	1.52	1.93	0.426	0.701
15	18.5	10.2	1.78	1.28	0.608	0.622	1.23	4.26	0.199	0.834
RWC, %			QP	K_{prp}	K_{fd}	V_t	QP	K_{prp}	K_{fd}	V_t
1	90.7	88.4	0.944	3.467	3.735	0.056	0.988	2.706	3.594	0.012
3	85.1	82.3	0.945	3.373	3.692	0.055	0.965	2.457	3.184	0.035
5	74.5	70.9	0.929	2.835	3.189	0.071	0.959	1.984	2.760	0.041
7	51.7	44.3	0.895	1.918	2.430	0.105	0.940	1.281	2.119	0.060
9	42.2	34.1	0.885	1.677	2.243	0.115	0.937	0.965	1.853	0.063
11	31.9	24.7	0.879	1.168	1.900	0.121	0.919	0.830	1.714	0.081
13	23.8	17.4	0.877	1.064	1.825	0.123	0.874	0.519	1.426	0.126
15	18.5	10.2	0.861	0.783	1.608	0.139	0.871	0.235	1.199	0.129

*1-'Alpha' variety; 2-'Zhuravka' variety; **-Day of simulated drought

From the 11th day, a decrease in all the basic indicators of the CFI curve was noted. The dynamics of growth of F_0 and F_{st} in 'Alpha' variety was 4.8 and 2.5 units day^{-1}. In the 'Zhuravka' variety, up to the 11th day of accounting, it was 1.8 and 3.7 units day^{-1}. The dynamics of decrease of F_{pl} and F_m indicators was 12.1 and 77.3 units day^{-1} in 'Alpha' variety, and 9.9 and 90.9 units day^{-1} in 'Zhuravka' variety. It should be noted that, given the dynamics of leaf RWC, its water-holding capacity in the process of drought development was different among the varieties. Leaves of 'Alpha' variety lost 4.8% of RWC day^{-1} of simulated drought. In the 'Zhuravka' variety, this figure was 5.2%.

Given that water holding capacity is an indicator of drought tolerance and lower moisture loss during drought is a sign of a higher level of adaptability (Buckley & Sack, 2019; Ding et al., 2020), 'Alpha' variety once again confirmed its superiority in terms of drought tolerance and our previous conclusions from the results. The same was confirmed by the indicator of leaf water potential (Lwp), when the decrease in the indicator in comparison of the first and 15th day of drought simulation was noted at the level of 60% in the variety 'Alpha' and 67% in the variety 'Zhuravka'.

Given that the leaf water potential (L_{wp}) in most species was 4–5 and (for prolonged drought was about 1 (Wang et al., 2019; 2022), the initial water status of 'Zhuravka' variety leaves on the first day of drought simulation can already be identified as initially stressful.

In accordance with the increase in stress load and changes in baseline parameters, other derived indicators of chlorophyll fluorescence also changed. The plant viability index (RF_d) was critical less than 1 (according to Huang et al. (2017); Kalaji et al. (2017) at RWC levels of 31.9% in 'Alpha' and 44.3% in 'Zhuravka' variety. There was also an increase in endogenous stress in terms of K_{ef} with a growth coefficient of 2.3 in 'Alpha' and 3.0 in 'Zhuravka' variety.

The ER index from the level of 0.830 under optimal conditions for most plant species (Kalaji et al., 2017) decreased compared to the first day of stress by 1.7 times in 'Alpha' and 3.8 times in 'Zhuravka' variety.

It is natural that against the background of an increase in photochemical quenching (QP), a decrease in the efficiency of primary reactions of photosynthesis (K_{prp}), a decrease in the fluorescence decay coefficient and F_v in both varieties of oilseed radish, the overall dynamics of fluorescence according to the V_t criterion was accelerated with a maximum value at the minimum level of RWC. It is emphasized that from the point of view of a systematic approach to the identification of drought tolerance, it was important to combine physiological and biochemical approaches based on a combination of assimilation processes and biochemical markers (Dellero et al., 2020; Monteoliva et al., 2021). As noted, one of these reliable markers was the proline content in the assimilative tissues of plants under the influence of stress of various types and intensities (Jurkonienė et al., 2023). The results of determining the proline content in the leaves of oilseed radish varieties under dynamic stress are shown in Figure 8.

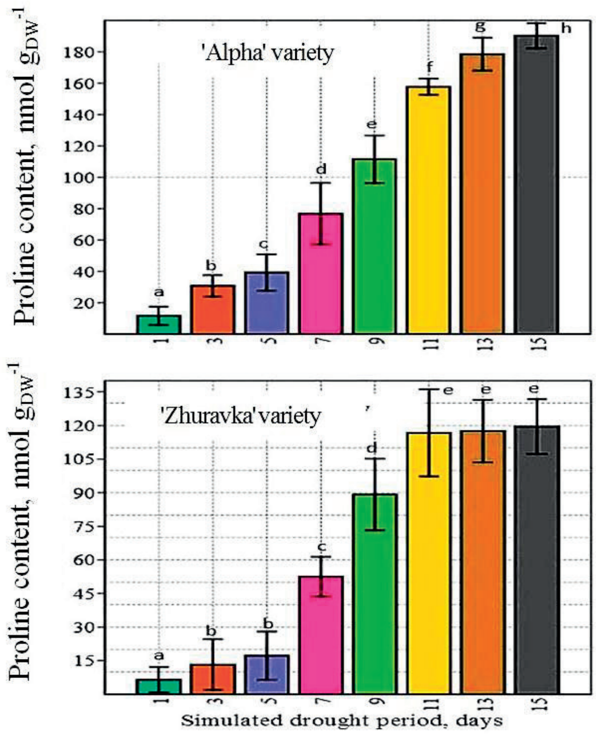


Figure 8. Proline content in leaves of oilseed radish varieties against the background of changes in leaf RWC in the flowering phase of plants (BBCH 61–67). Note: Different letters indicate values that differed significantly for level *p<0.05*

According to the determined proline content under the influence of drought in different types of cruciferous crops (Batool et al., 2022; Jurkonienė et al., 2023; Kashyap et al., 2023), it was found that oilseed radish has a similar mechanism and intensity of proline response to spring and winter rape, various types of mustard, including its wild relatives.

An increase in the proline content in leaf tissues of both oilseed radish varieties was found. The gradient of this growth was 11.88

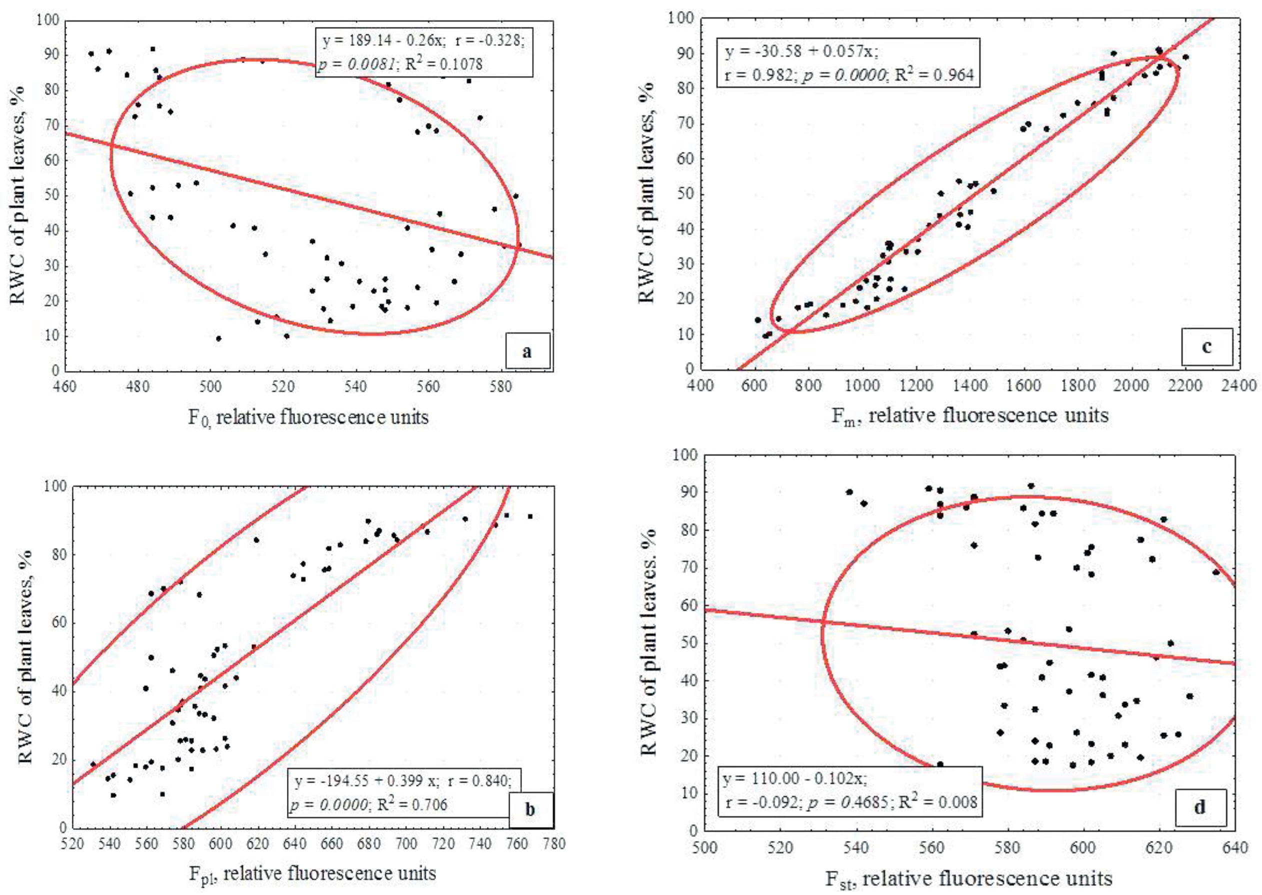
nmol g_{DW}^{-1} per day in 'Alpha' variety and 7.40 nmol g_{DW}^{-1} per day in 'Zhuravka' variety. The dynamics of proline concentration had a number of peculiarities in the varieties. For the 'Alpha' variety, which was determined to be more drought-resistant, a significant difference in proline concentration was noted throughout the entire stage of its accounting. For 'Zhuravka' variety, the difference was significant for the periods between the first and third days of recording and between the fifth and eleventh days. Given that for drought-tolerant cruciferous species, proline concentration had a high direct correlation with stress intensity (Kashyap et al., 2023; Sakpal et al., 2023), 'Zhuravka' variety had a physiologically distant proline biochemical response to drought. This led to the determined effect of a significantly greater reduction in both basic and derived indicators of chlorophyll fluorescence. The results of the study confirmed the findings of Tang et al. (2019), Singh et al. (2022), Netshimbupfe et al. (2022) that both the intensity of proline accumulation in assimilative tissues and a positive and significant increase in its concentration with increasing overall stress during the period of active plant growth are important for drought-tolerant species. At the same time, the nature of the change in the stress load indicator should have a direct relationship (Figure 9, Table 4).

Table 4. Correlation dependence between basic CFI curve indicators, RWC (leaves, %) and PC (leaves, nmol g_{DW}^{-1}) in oilseed radish varieties for flowering phase (BBCH 61-67)

Indicators	RWC	PC	RWC	PC	RWC	PC
	In the dataset for both varieties (*N=64*)		'Alpha' variety (*N=32*)		'Zhuravka' variety (*N=32*)	
F_0	-0.341	0.166	-0.894*	0.950*	0.263	-0.292
F_{pl}	0.875*	-0.716*	0.939*	-0.888*	0.835*	-0.767*
F_m	0.991*	-0.831*	0.995*	-0.962*	0.996*	-0.968*
F_{st}	-0.125	0.214	-0.971*	0.974*	0.267	-0.288

*significant at $p<0.05$ level

According to the calculations, a significantly different response of varieties to changes in leaf RWC was found in terms of the dynamics of F_0 and F_{st} formation and proline content.



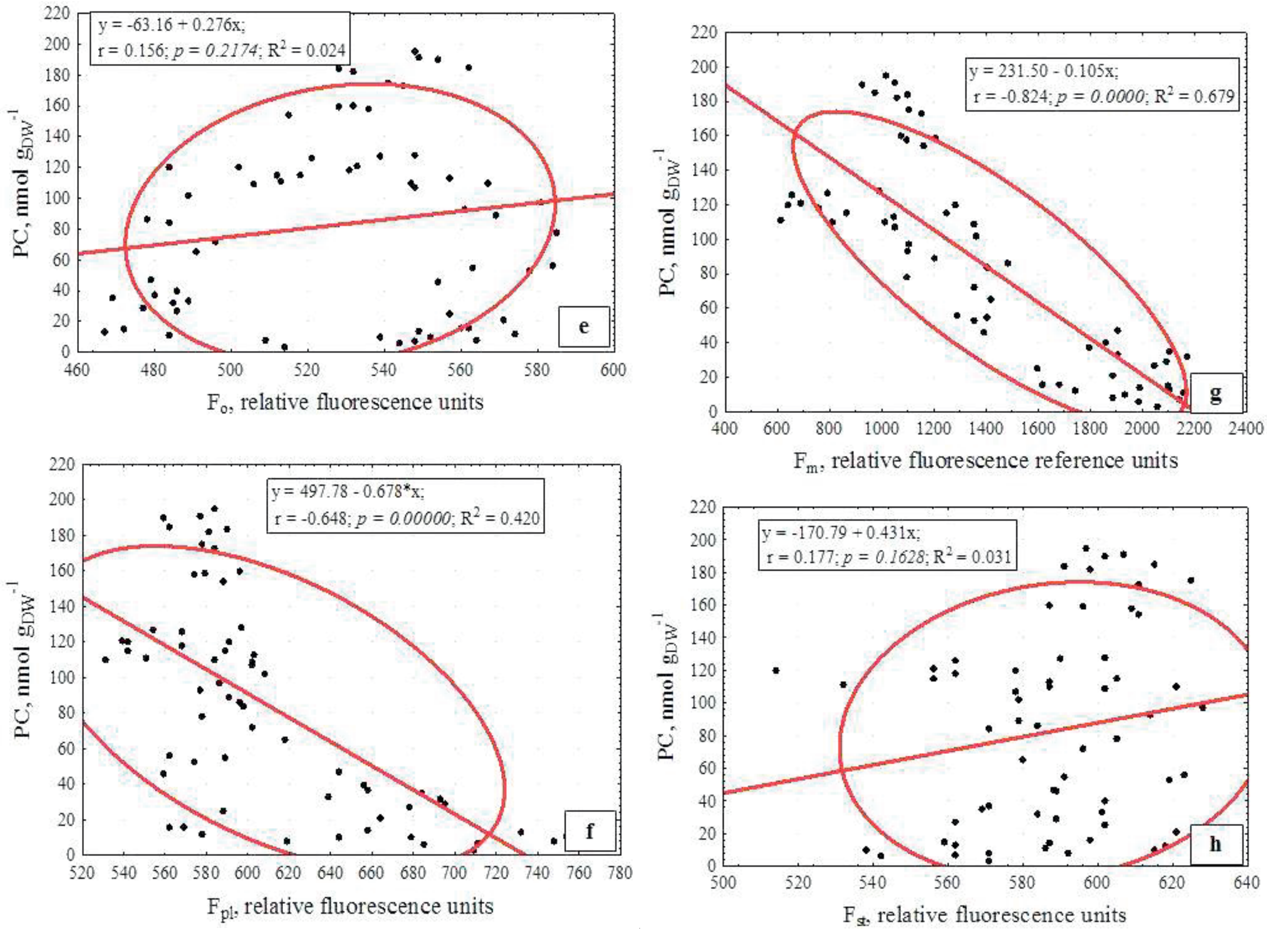


Figure 9. Graphical representation of the regression between the basic CFI curve indicators and RWC (leaves, %) and PC (leaves, nmol gDW^{-1}) values (for the combined data set by varieties, *N=64*)

This ensured the preservation of a certain direction of the relationships and a decrease in its closeness in a single analysis data set for both varieties (Figure 9, a, d, e, h). When assessing within individual varieties of different stress resistance, the direction of correlation was different.

Thus, the coefficient of determination (d_{xy}) in the more drought-resistant 'Alpha' variety of the value of the formed indicators F_0 and F_{st} depending on the RWC of leaves was inverse and amounted to 79.9% and 94.3%, respectively.

For 'Zhuravka' variety, this relationship was direct with a level of determination of 6.9% and 7.1%. Similar differences were found in the relationship between the major indicators of chlorophyll fluorescence and proline content (Table 4).

These results confirmed the conclusions about the difference in the photochemical and biochemical stress response system of plants with different degrees of stress resistance (Wani et al., 2017).

CONCLUSIONS

The feasibility and methodological efficiency of using chlorophyll fluorescence and proline content in leaves to assess plant drought tolerance both in terms of phenological development of plants and for the identification of drought-resistant genotypes were established.

The drought resistance of oilseed radish plants was minimal in the flowering phase and tended to be dynamically increasing with the drought effect according to the criterion of leaf SWC reduction, which had an inverse relationship with the values of F_0 and F_{st} and a close direct relationship with the values of F_{pl} and F_m. The model of a drought-tolerant plant predicted low growth rates of F_0 and F_{st} with low rates of decrease of F_{pl} and F_m on the chlorophyll fluorescence curve.

For the drought-tolerant oilseed radish variety, a steady increase in the concentration of proline in leaf tissues was observed starting from the first stress period with a graded growth index of at least 10 nmol g_{DW}^{-1} day^{-1} in the period from the state of no stress to severe drought conditions.

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