

PHYSIOLOGICAL PARAMETERS AND VEGETATIVE BEHAVIOR OF BIOLOGICAL GROWN HEAD LETTUCE TYPE (*LACTUCA SATIVA* L. VAR. *CAPITATA* L.)

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Abstract

The climate change that has occurred in the last three decades is also present for the region of Plovdiv in Bulgaria. Increase of annual average air temperature and temperature values during winter and early spring has been recorded in comparison to the (1961-1990) referent period in Plovdiv region. The lettuce development was researched in a greenhouse in the Agricultural University- Plovdiv. During the lettuce growth six different variants were used: no fertilizer, one chemical, and four organic fertilizers. The effect of the different organic fertilizers was studied through physiological parameters and vegetative behavior of plants. This paper analyzed changes in functional activity of the plant photosynthetic apparatus and productivity of variants with different fertilizers in an unheated greenhouse. The ratio between photosynthetic active radiation (PAR) and quantum yield ($qY-Fv/Fm$) of PS II was more effective in dark-adapted leaves for the organic fertilizer variants, compared to the no fertilizer variant. No significant difference was observed in the values of the minimal fluorescence F_o in reaction centers of PS II after the dark-adaptation of leaves from the different fertilizer variants. It was estimated the higher value of the chlorophyll content index (CCI) for organic and chemical fertilizers compared to the no fertilizer variant. The main biometric parameters were studied.

Key words: photosynthetic activity, chlorophyll content index, lettuce, vegetative behavior, greenhouse.

INTRODUCTION

In recent years, with the intensification of extreme phenomena of meteorological origin, the advancement of agricultural science and the desire of people to produce qualitative and safe food with minimal risk to the environment, biological farming technologies have aroused scientific interest. Production without mineral fertilizers is important for the environment protection, for the balance and fertility of the soil, as well as for human health. The lettuce is a vegetable, intended only for fresh consumption, which requires good taste and purity of production. Vitamins A, B, C, D, and E can be found in the leaves of the species (Fogg, 1983). The vegetable is one of the main components from the dietary menu and the table in Bulgaria.

The resistance of the species to low temperatures and the duration of the period up to their typical leaf mass reached growth stage make it preferred both for autumn-winter production in unheated facilities and for early spring cultivation. Both the higher temperatures

and the changes in the humidification conditions in the country (Marinova et al., 2018; Alexandrov et al., 2004), and the studied area (Georgieva et al., 2017), registered in the recent decades, affect the specific meteorological conditions, the growth and development of the different production (Popova et al., 2014) and types of lettuces. It is necessary to specify the varieties and the fertilization. Therefore, an experiment in polyethylene greenhouses with a type of lettuce was set (type Head lettuce, variety “Winter butterhead”) with six different variants of organic (biological) fertilization, namely: no fertilization; fertilization by means of one chemical; and fertilization by four organic fertilizers.

There are studies on the interaction of different factors on the physiological status of plants (Shopova & Cholakov, 2014). The physiological condition of plants and effect of various stressful factors thereon have been studied using chlorophyll fluorescence properties by many researchers (Mathur et al, 2014; Kalaji et al., 2016). Chlorophyll

fluorescence is a non-invasive measurement of photosystem II (PSII) activity and is a commonly used technique in plant physiology. The sensitivity of PSII activity to abiotic and biotic factors has made this a key technique not only for understanding the photosynthetic mechanisms but also as a broader indicator of how plants respond to environmental change (Murchie & Lawson, 2013). The fluorescence is emitted mainly from chlorophyll *a* of PSII and reflects the primary processes of photosynthesis by light absorption, distribution and transfer of excitation energy and photochemical reactions in PSII. Because of the functional relation of PSII with other components of the photosynthetic apparatus of the chlorophyll fluorescence, it is seen as a proxy for the state of the integral photosynthetic process and the plant organism as a whole (Roháček, 2002). Chlorophyll fluorescence, among others, has been satisfactorily used for monitoring leaf health status in lamb's lettuce (Ferrante & Maggioro, 2007) and storage potential of iceberg lettuce (Schofield et al., 2005).

The device Chlorophyll Content Meter is useful for improving nitrogen and fertilizer management, and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Opti-Sciences 2002; Richardson et al., 2002).

The aim of the present study was to monitor the reaction of the lettuce (type Head lettuce, variety "Winter butterhead") to six different fertilization variants by analyzing the temperature conditions and measuring the main parameters of productivity and photosynthetic activity.

MATERIALS AND METHODS

The experiment was conducted on the experimental field of the Agricultural University of Plovdiv in 2018-2020 in unheated greenhouses on alluvial meadow soil (Mollic fluvisol, FAO 2006). The soil texture is sandy clay loam to clay loam, despite the small amount of total carbonates (2-3%), the soil reaction is slightly alkaline pH (H₂O) - 7.7-8.0

(Valcheva et al., 2015). The same authors found a high amount of exchange bases (Ca²⁺+Mg²⁺ - 20-30 meq/100 g soil) in the composition of the soil sorption complex, and a low content of nitrogen, phosphorus and potassium. The importance of the organic matter of the soil for its fertility is indisputable. However, the nitrogen bound in the organic matter remains hidden in this indicator. The nitrogen in organic form, which is over 95.0% of total soil nitrogen is the basis of soil fertility. Organic nitrogen is the source that supports the plants throughout the growing season and ensures an even supply of nitrogen to the plants. The active fraction of soil nitrogen varies with different soil types and depends on a number of factors - degree of cultivation, field history (previous crops in the crop rotation, fertilization system), biotic and abiotic soil characteristics and some environmental factors, mainly temperature and humidity.

The head lettuce type plants (*Lactuca sativa* L. var. *capitata* L., variety 'Winter butterhead') were planted on 8th of November in polyethylene greenhouses in 4 rows according to the scheme 70+30+30+30/30 cm with a profile of the soil surface a high level bed (100+60cm.) The experiment was based on the block method with four repetitions, using 28 plants per repetition, and a plot size of 3.36 m². Organic seeds were provided for seedling production using container technology with 150-hole Styrofoam boards in the following combination - organic seeds - 80.0%, Perlite - 20.0%, Lumbricompost for bioproduction of seedlings (Kostadinov & Filipov, 2013). Several variants were tested: 1. Control (non-fertilization); 2. NPK (mineral fertilization); 3. Italpollina; 4. Arkobaleno; 5. LC (Lumbricompost); 6. Ekoprop NX. The granular fertilizers were introduced as basic fertilization, with soil pre-transplantation at the following norms: N-12.5 kg/da, P₂O₅-1.25 kg/da, + K₂O-4.75 kg/da, Italpollina-25 kg/da, Arkobaleno - 100 kg/da, and Lumbricompost - 400 l/da. The liquid bio fertilizer Ekoprop NX was applied by double treatment in a dose of 100 g/da, before planting - in the 5th leaf seedling phase, and 10 days later on, after the adaptation to the soil. The remaining bio fertilizers are granulated and introduced into the soil before the last tillage and before

planting the seedlings. The biometric measurements were taken three times at one-week intervals in stage-typical leaf mass reached.

Chlorophyll fluorescence imaging. The Chlorophyll fluorescence of the lettuce leaves was measured using a portable device PAR-FluorPen FP 110/D manufactured by Photon Systems Instruments Ltd., Czech Republic. The fluorescence measurement protocol uses short (30 μ s) measuring flashes to measure zero level fluorescence (F_0) followed by a strong saturating flash (duration 0.8 s, intensity about 3000 μ mol $m^{-2} s^{-1}$) to measure the maximum fluorescence (F_m). Three strong flashes of saturating light probed the effective quantum yield (Qy) of PSII during the actinic light exposure (Maxwell & Johnson, 2000; Nedbal et al., 2000). Light Meter for direct digital readouts of Photosynthetically Active Radiation (PAR) in the range from 400 to 700 nm, the span in which plants use energy during photosynthesis. PAR is measured as Photosynthetic Photon Flux Density (PPFD), which is indicated by units of quanta (photons) per unit time per unit surface area. The chlorophyll fluorescence transients were measured on the same day in the morning. The periods of measurement were between the end of March and the beginning of April, when the plants were in their typical leaf mass reached growth stage. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The numeric value of each parameter (F_v/F_m , F_0 , PAR) was determined by integrating it over the measured leaf area.

Physiological estimate of the chlorophyll content index (CCI). The Chlorophyll content index of the leaves was measured using a portable apparatus CCM 200 plus manufactured by Opti-sciences, Inc., NH, USA. The physiological assessment was carried out *in vivo* on the field. The measurements were taken on three dates from a sample of leaves at their typical leaf mass reached growth stage. The periods of the measurements were between the end of March and the beginning of April. 20 leaf measurements in the central part of the leaves were taken for each variant (in each of the repetitions).

Statistical evaluation of the results: The statistic processing of the data was performed by applying the mono-factorial dispersion analysis (Dimova & Marinkov, 1999).

RESULTS AND DISCUSSIONS

Physiological parameters

The mean value of the initial fluorescence (F_0) of the oxidized reaction centers of PSII was highest in the mineral fertilization variant, and lowest in the Italpollina and Arkobaleno organically fertilization variants (Table 1). For this parameter, significant differences in the average values of the different fertilization variants were not calculated. In relation to the studies of Zlatev & Kolev, 2012 and Chen et al., 2018, who believe that a higher value of F_0 is associated with high temperature stress, we can conclude that the temperature conditions in the greenhouse do not lead to a stress response in the plants. For parameter Qy (F_v/F_m), a statistically significant lowest value was registered for the control plants - 0.790 and a highest value was read for the biological fertilization variant Italpollina - 0.812. Higher values were also reported for the all variants compared to the control (Table 1). The comparative characteristic made by dates of measurements shows the largest difference between the unfertilized variant and the variants with organic fertilizers on the second measurement date. The mean value of Qy for all variants in this study indicates the presence of moderate stress in the photosynthetic activity of the plants, most pronounced in the control variant. It was confirmed from the average value of the ratio F_v/F_m or the quantum yield Qy of the different variants, which was close to the normal for healthy leaves - 0.83 (Demmig & Björkman, 1987). The measured photosynthetically active radiation (PAR) is higher on the first two dates, which is associated with the higher daily temperatures and the increased solar radiation compared to the atmospheric conditions during the third reporting date. The higher PAR values on the first two dates are associated with a lower Qy value, and this reduction should not be associated with photoinhibition due to low PAR values. The ratio between photosynthetic active radiation (PAR) and quantum yield (qY -

Fv/Fm) of PS II in dark-adapted leaves was more effective for the organic fertilizer variants Italpollina and Ekoprop, as well as for the variant with mineral fertilization (Table 1).

In parallel with the readings of some indicators of the chlorophyll fluorescence of the leaves, the chlorophyll index - Chlorophyll Content Index (CCI) was measured (Table 2). The lowest mean CCI value was estimated for the non-fertilized control variant, with few exceptions this dependence being maintained

for all three dates of measurement. The leaves of the variants fertilized by organic fertilizers Ekoprop have the highest CCI. The significance difference only for organic fertilizer variant Ekoprop compared to the control variant was calculated. The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy ($r = 0.648$) which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants.

Table 1. Chlorophyll fluorescence parameters of the plant leaves for the head lettuce type (*Lactuca sativa* L. var. *capitata* L.) variety 'Winter butterhead' in an unheated greenhouse, averaged for the period 2018-2020 year

	Control	NPK	Italpollina	Arkobaleno	LC	Ekoprop
f_0	4513	3531	3561	4858	4844	5109
f_0	5209	5715	5041	4400	5449	5215
f_0	4626	5226	4882	4848	4335	4149
	4782.6	4823.9 n.s.	4494.7 n.s.	4702.0 n.s.	4875.9 n.s.	4824.3 n.s.
Qy=Fv/Fm	0,813	0,810	0,803	0,807	0,810	0,807
Qy=Fv/Fm	0,760	0,787	0,800	0,780	0,792	0,783
Qy=Fv/Fm	0,797	0,825	0,833	0,828	0,827	0,833
	0.790	0.807*	0.812 **	0.805*	0.809 *	0.808**
PAR	110,0	79,7	97,0	121,0	145,3	135,0
PAR	124,0	135,0	130,0	153,3	183,0	175,3
PAR	145,3	110,0	86,7	83,3	78,0	68,3
	126.4	108.2 n.s.	104.6 n.s.	119.2 n.s.	135.4 n.s.	126.2 n.s.

LSD
 $p = 0.05^*$ F₀ Qy PAR
 $p = 0.01^{**}$ 557.1 0.0015 27.6
 $p = 0.001^{***}$ 779.3 0.0020 36.9
 1054.5 0.0022 48.5
n.s. - no significance difference

Table 2. Chlorophyll content index (CCI) of the leaves for the head lettuce type (*Lactuca sativa* L. var. *capitata* L.) variety 'Winter butterhead' in an unheated greenhouse averaged for the period 2018-2020 year

Date of estimate / Variants	Control	NPK	Italpollina	Arkobaleno	LC	Ekoprop
first date	4.85	4.87	5.42	6.68	5.48	6.00
second date	4.36	5.46	4.90	4.60	5.16	5.56
third date	4.90	6.34	4.82	5.02	5.96	5.68
mean value	4H.70	5.55 n.s.	5.03 n.s.	5.43 n.s.	5.53 n.s.	5.75*

LSD
 $p = 0.05^*$ 0.91
 $p = 0.01^{**}$ 1.20
 $p = 0.001^{***}$ 1.56
n.s. - no significant difference

Vegetative behavior

Head lettuce type plants are characterized by a faster rate of growth and development and form a bigger vegetative mass, which can also be seen from the readings of the 'Winter butter head' variety. At the moment of the first reading, the fresh mass of the whole plant was

relatively high, meeting the market requirements, though (Table 3). The largest mass was formed after mineral fertilization with Italpollina, Arkobaleno and Ekoprop- between 391.25 g and 452.86 g. The yield in fresh mass of the other variant with organic fertilization is 363.62 g.

Table 3. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the first biometric measurement, for the experimental period 2018-2020 year

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
	year			year			year			diameter, mm			mass, g		
	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average	13.03. 2019	21.03. 2020	average
1. Control	298.42 ^{n.s.}	202.16 ^{n.s.}	250.29	30.12 ^{n.s.}	28.91 ^{n.s.}	29.52	34.06 ^{n.s.}	32.08 ^{n.s.}	33.07	21.57 ^{n.s.}	20.00 ^{n.s.}	20.78	21.50 ^{n.s.}	19.24 ^{n.s.}	20.37
2. NPK	412.25	354.08	383.17	37.17	35.74	36.45	38.17	35.66	36.91	25.55	23.20	24.38	28.50	26.33	27.41
3. Italpollina	426.17	356.33	391.25	37.25	36.83	37.04	38.50	35.99	37.24	24.58	23.98	24.28	31.50	26.91	29.21
4. Arkobaleno	447.33	369.58	408.46	37.50	37.66	37.58	39.23	36.24	37.73	24.74	25.29	25.02	32.33	28.66	30.50
5. LC	391.17 ^{n.s.}	336.08 ^{n.s.}	363.62	36.92	35.58	36.25	37.84	35.49	36.66	25.28	23.07	24.17	27.09	24.99	26.04
6. Ekoprop NX	480.39	425.33	452.86	42.33	39.91	41.12	39.30	38.91	39.11	25.60	25.48	25.54	35.28	31.49	33.38
GD 95% = (+); (-)	100.25	92.23		6.35	3.24		1.5	2.49		2.59	2.41		6.38	7.48	6.93
GD 99% = (++) ; (-)	138.85	127.74		8.79	4.50		2.09	3.45		3.59	3.34		8.63	10.36	9.495
GD 99.9% = (+++); (---)	191.57	176.24		12.14	6.20		2.88	4.76		4.96	4.61		12.19	14.3	13.245

n.s. - no significance difference

Relatively small differences were reported in the diameter of their rosette. The largest diameter is reported for for Ekoprop and Arcobaleno - 39.11 and 37.73 cm, respectively. The variants with organic fertilization have formed a rosette with a diameter between 36.62 and 39.11 cm.

The plants had formed a relatively smaller number of leaves. The plants, fertilized with Ekoprop had the highest number of leaves - 41.12, followed by Arcobaleno with 37.58. The other variants formed between 29.52 and 41.12 leaves.

Larger differences were observed with the indicators of the stem. The differences in diameter were significant. It was the largest with Ekoprop - fertilization - 25.54 mm and with Arkobaleno- 25.02 mm. The highest mass value had the stem with the Ekoprop variant of fertilization - 33.38 g, followed by the resulting stem in the case of using Arkobaleno - 30.50 g. With the smallest mass of the stem were the unfertilized plants and Lumbricompost, 20.37 and 26.04 g, respectively.

During the two experimental years, with the exception of the fresh mass of plants fertilized with Lumbricompost, the statistical difference between the tested variants was proved.

The second reading revealed that the plants had continued to grow, albeit slowly (Table 4). The fresh mass of the whole plant had increased compared to the previous moment of measurement. The largest were the plants, fertilized with Ekoprop - 524.14 g. The options with organic fertilization - Italpollina, Lumbricompost and Arkobaleno - were close in value to conventional fertilization and ranged from 476.96 g to 481.62 g.

There were small differences between the variants of fertilization in the diameter of the plant rosette. It ranged from 34.92 mm for the control variant (no fertilization) to 39.56 mm for the variant of fertilization with Ekoprop. The values of the stem indicators were equalized. The diameter of the stem ranged from 23.06 mm for the control variant (no fertilization) to 30.53 mm for the variant of fertilization with Ekoprop.

Table 4. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the second biometric measurement, for the experimental period 2018-2020 YEAR

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
	year			year			year			diameter, mm			mass, g		
	year			year			year			year			year		
	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average	20.03. 2019	21.03. 2020	average
1. Control	358.50 ^{n.s.}	205.75 ^{n.s.}	282.13	40.09 ^{n.s.}	30.58 ^{n.s.}	35.33	36 ^{n.s.}	33.83 ^{n.s.}	34.92	24.03 ^{n.s.}	22.08 ^{n.s.}	23.06	33.59 ^{n.s.}	20.83 ^{n.s.}	27.21
2. NPK	505.58	387.41	446.50	46.50	39.66	43.08	38.83	37.49	38.16	27.74	25.75	26.75	39.50	36.66	38.08
3. Italpollina	539.84	414.08	476.96	46.58	41.66	44.12	38.92	38.08	38.50	31.39	27.24	29.32	39.75	39.75	39.75
4. Arkobaleno	542.42	420.83	481.62	49.61	41.91	45.76	40.34	38.33	39.33	32.35	28.36	30.36	40.50	41.33	40.91
5. LC	496.08	365.99	431.04	46.42	39.24	42.83	38.69	37.49	38.09	27.57	25.66	26.61	39.50	36.49	38.00
6. Ekoprop NX	607.45	440.83	524.14	49.83	42.91	46.37	40.61	38.50	39.56	32.49	28.58	30.53	40.61	41.74	41.17
GD 95% = (+); (-)	121.74	129.33		5.37	6.67		2.72	2.89		2.58	3.35		2.32	15.65	
GD99% = (++); (-)	168.61	179.12		7.44	9.24		3.77	4.01		3.58	4.65		3.21	21.68	
GD 99.9% = (+++); (---)	232.62	247.12		10.26	12.75		5.20	5.53		4.94	6.41		4.43	29.91	

n.s. - no significance difference

The mass of the stem showed comparatively small differences between the variants. It was slightly higher for Ekoprop, Arkobaleno and Italpollina fertilization, namely: 41.17 g, 40.91 g and 39.75 g respectively.

The statistical difference between the tested variants by experimental years has been proven. Plant growth was registered at the moment of the third harvest (Table 5). The fresh mass of the whole plant had increased slightly. The largest were the plants, fertilized with Ekoprop - 531.47 g, exceeding the mineral fertilized ones by 52.26 g. The other variants with organic fertilization were similar in size to conventional fertilization.

New leaves had been formed in all variants of fertilization, ranging from 1.42 after Italpollina

to 3.90 after mineral fertilization. The total number of leaves was between 38.27 for the unfertilized plants to 48.72 for the ones, fertilized with Ekoprop.

Leaf growth was also registered, which caused the formation of a larger leaf rosette, with a diameter, ranging from 33.66 mm to 39.67 mm. In most variants, the mass had increased significantly, while its diameter of the stem had grown less. The plants fertilized with Ekoprop had the largest mass - 50.85 g, while the ones, fertilized with mineral fertilization and Italpollina were with a mass of 48.95 and 48.12 g. This trend is valid for the diameter as well, with Ekoprop and Arkobaleno fertilized plants having the largest diameters. The diameter had increased by 2-3 mm, but the

differences between the variants were significant - 4-9 mm. The statistical difference

between the tested variants was proved for the two experimental years.

Table 5. Vegetative behavior of the head lettuce type, variety 'Winter butterhead' in the third biometric measurement, for the experimental period 2018-2020 YEAR

Variant	fresh mass of the whole plant, g			leaves, number			leaf rosette diameter, cm			stem					
	year			year			year			diameter, mm			mass, g		
	28.03.2019	21.03.2020	average	28.03.2019	21.03.2020	average	28.03.2019	21.03.2020	average	28.03.2019	21.03.2020	average	28.03.2019	21.03.2020	average
1. Control	409.07 ^{n.s.}	207.49 ^{n.s.}	308.28	40.25 ^{n.s.}	36.29 ^{n.s.}	38.27	36.59 ^{n.s.}	30.74 ^{n.s.}	33.66	25.54 ^{n.s.}	24.03 ^{n.s.}	24.79	34.58 ^{n.s.}	25.83 ^{n.s.}	30.21
2. NPK	559.08	399.33	479.21	49.42	44.54	46.98	39.50	35.58	37.54	30.49	27.74	29.12	56.50	41.40	48.95
3. Italpollina	578.00	424.74	501.37	46.84	44.25	45.54	39.75	35.49	37.62	31.50	31.39	31.45	53.92	42.33	48.12
4. Arkobaleno	580.09	437.25	508.67	51.17	46.54	48.85	40.50	37.66	39.08	32.36	32.35	32.36	54.34	42.57	48.45
5. LC	555.92	366.16	461.04	46.50	42.87	44.69	39.50	36.08	37.79	30.08	27.57	28.82	50.17	38.31	44.24
6. Ekoprop NX	616.86	446.08	531.47	50.50	46.95	48.72	41.25	38.08	39.67	35.28	32.49	33.88	57.56	44.14	50.85
GD 95% = (+); (-)	138.15	156.67		5.99	6.74		2.32	2.97		3.39	2.58		14.06	10.98	
GD 99% = (++) ; (-)	191.33	216.99		8.30	9.34		3.21	4.11		4.70	3.58		19.47	15.21	
GD 99.9% = (+++) ; (-)	263.97	299.38		11.46	12.88		4.43	5.67		6.49	4.94		26.83	20.99	

n.s. - no significance difference

CONCLUSIONS

The ratio between photosynthetic active radiation (PAR) and quantum yield (qY-Fv/Fm) of PS II in dark-adapted leaves was more effective for the organic fertilizer variants Italpollina and Ekoprop,

The leaves of the variants fertilized by organic fertilizers Ekoprop have the highest CCI.

The values of the chlorophyll index are in a positive correlation with the values of the quantum yield-Qy, ($r = 0.648$) which proves the inducing effect of the organic fertilizers on the photosynthetic activity of the plants.

Organic fertilizers are able to meet the need for essential nutrients when growing lettuce in polyethylene greenhouses. All variants with organic fertilization comply with the quality standard from the beginning to the end of

harvesting. The variant with organic fertilization with Lumbricompost form plants close in their average mass to those, grown after conventional fertilization with mineral fertilizers, and the plants grown after organic fertilization with Italpollina, Arkobaleno, and Ekoprop surpass it.

Under the meteorological conditions of the experiment, biological fertilization does not require photosynthetic activity of plants compared to those found after mineral fertilization.

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