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# CAULIFLOWER'S RESPONSE TO DROUGHT STRESS $^{\ast}$

# REAKCJA KALAFIORA NA STRES SUSZY

#### Summary

**Background.** Cauliflower has high water requirements due to the heavy weight of its leaves, so drought stress is one of main factors affecting plant growth and yield. The aim of this study was to determine the impact of drought stress on the physiological parameters of the cauliflower plant. **Material and methods.** Cauliflower plants cv. 'Sevilla' F<sub>1</sub> were grown in containers filled with a peat substrate of different water contents i.e. 20%, 30%, 40%, 50% and 60% of field capacity. These parameters were measured: net photosynthetic rate, transpiration rate, chlorophyll fluorescence, stomatal conductance, intercellular CO<sub>2</sub> concentration, and electron transport rate. Measurements were taken at the 5-leaf phase after 9 days of drought.

**Results.** The lowest moisture level was observed at the substrate water content (SWC) of 20% of the field capacity and as a result, the plants grown in this substrate absorbed the smallest amount of water. At a 20% SWC, cauliflower plants showed the lowest photosynthetic rate, transpiration rate and stomatal conductance, while the  $CO_2$  content in substomatal cavities and electron transport rate were the lowest. However, there were no significant differences in chlorophyll fluorescence between all SWC levels.

**Conclusions.** There was a significant correlation between the substrate water content and the physiological response of the plants to drought stress. Drought stress was delayed as the substrate water content increased. On the other hand, severe water deficit resulted in faster plant response to drought stress.

Key words: Brassica oleracea L. var. botrytis subvar. cauliflora DC., transpiration, photosynthesis, chlorophyll fluorescence, stomatal conductance, electron transport rate

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# Introduction

Drought is one of the most important stress factors affecting plants. Soil water content determines plant growth and development, yield quality and quantity, and consequently, cultivation profitability (Mirabad et al., 2013). Cauliflower is one of the most popular field-grown vegetables. This crop has high water requirements due to its large weight of leaves.

Drought leads to physiological, biochemical and molecular changes in plants (Ashraf and Harris, 2013; Yordanov et al., 2000). One of the first responses of a plant to drought stress is reduced transpiration caused by stomatal closure, which results in higher CO<sub>2</sub> content in substomatal cavities (Harb et al., 2010; Sikuku et al., 2010).

Many authors point to the fact that limitation of photosynthesis may also be nonstomatal and related to metabolic changes (Flexas et al., 2008; Galmés et al., 2011; Warren, 2008). According to Galmés et al. (2007a), the impact of stomatal and nonstomatal limitation depends on drought stress intensity. Stomatal conductance is the indicator that determines the influence of stomatal and nonstomatal limitations on photosynthesis (Xu and Zhou, 2008). It is strictly correlated with CO<sub>2</sub> content in substomatal cavities (C<sub>i</sub>) and photosynthesis (Flexas and Medrano, 2002). As stated by Razavi et al. (2008), drought stress may lead to changes in chlorophyll fluorescence kinetics and its measurements indicate photochemical activity of the photosynthetic apparatus. The  $F_v/F_m$  ratio is a parameter which determines the likelihood of photosystem (PSII) damage and occurrence of photoinhibition.

There have been numerous studies on the impact of drought stress on physiological changes in many plant species (Campos et al., 2014; Galmés et al., 2007b; Miyashita et al., 2005; Warren, 2008), but there have been few similar studies on cauliflower.

The aim of this study was to determine the impact of drought stress on the intensity of photosynthesis, transpiration and other physiological parameters of cauliflower plants at different substrate water contents (SWC) and at two photosynthetic photon flux densities (PPFD).

#### Materials and methods

The study was conducted in two cultivation cycles in 2012 and 2013, in a growth chamber at the Poznań University of Life Sciences, Poland. The treatments differed in the substrate water content (SWC), i.e. 20%, 30%, 40%, 50% and 60% of field capacity. In preliminary studies, measurements were taken at several levels of PPFD and it was found that the reaction of the plant to the deepening stress at low PPFD was difficult to detect. Therefore, in the present study measurements were taken at two levels of photosynthetic photon flux density (PPFD), i.e. 1000 and 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>.

#### Plant material and growth conditions

The object of the study was 'Sevilla'  $F_1$  (Bejo Zaden) cultivar of cauliflower (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora* DC.). Cauliflower seedlings were produced in 0.5 dm<sup>3</sup> pots filled with peat substrate for growing cruciferous vegetables

(Kronen-Klasmann). When the seedlings had 3-4 leaves, they were transplanted to bigger containers (20 dm<sup>3</sup>), filled with the substrate of different SWC (20%, 30%, 40%, 50% and 60%). The SWC was determined by the water capacity curve dependent on the 14 combinations of perlite to peat ratio. On the curve, the points were found that indicated the proportion of peat and perlite to achieve adequate water capacity.

Before planting, minerals were supplemented to the maximum optimum level (in milligrams per 1 dm<sup>3</sup> of substrate): N-NO<sub>3</sub> – 250, P – 200, K – 600, Ca – 1600, Mg – 160 + microelements. For this purpose, 2 g·dm<sup>-3</sup> PG-Mix (Yara Poland), 0.7 g·dm<sup>-3</sup> of potassium sulfate and 4 g·dm<sup>-3</sup> dolomite were used. Additionally, during the growing period the plants were fed with complex fertilisers, i.e. calcium nitrate and Kristalon blue (Yara Poland).

By the time measurements were taken the plants had been kept in a growth chamber at a temperature of 18/16°C (day/night), 60% relative air humidity (RH) and 350 ppm CO<sub>2</sub> content. The photoperiod was 16 h and the PPFD was 150  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>. At the stage of 5 leaves the plants were watered to 100% of field capacity (FC). Afterwards they were grown for 9 days without irrigation, at a temperature of 25°C (while the other parameters did not change).

#### Measurements

Measurements of photosynthesis intensity, transpiration and chlorophyll fluorescence were taken.

Before plant measurements, the SWC was determined with the weight method. Net photosynthetic rate (A), transpiration rate (E), stomatal conductance ( $g_s$ ), electron transport rate (ETR) and intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) were measured using LCpro+ system (ADC BioScientific), which automatically set levels of CO<sub>2</sub> (360 ppm), PPFD (1000, 1700 µmol·m<sup>-2</sup>·s<sup>-1</sup>), RH (30%) and air temperature (25°C), depending on the program selected. The measurements were made when all the parameters stabilised. They were taken on one leaf of each plant, from the central part of it.

The plant stress caused by the factors under study was determined by measuring chlorophyll fluorescence (OS1-FL Fluorometre of Opti Science). Maximum photochemical efficiency of PSII ( $F_v/F_m$ ) was determined after 8 h of darkness (three measurements in each replication, n = 12). Measurements in light (Y = Yield) were performed directly after photosynthesis intensity and were measured on the same leaves.

All measurements at the PPFD of 1000 and 1700  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> were carried out on the same plants. The amount of evaporated water in the process of evapotranspiration was determined by gravimetric method, using mass measurements of containers with plants in the 0 and 9 day of stress.

#### Statistical analysis

The experiment was established as one-factor design, in four replicates (one plant in each treatment) in each cycle. The mean results of two cycles are presented. The significance of the SWC to the physiological parameters was determined with the ANOVA. Differences between the means were estimated with the Newman-Keuls test at a significance level of P = 0.05.

# **Results and discussion**

Lower SWC values led to faster occurrence of drought stress. The lowest moisture was observed at a SWC of 20% FC and as a result, the plants grown in this substrate absorbed the smallest amount of water (Table 1). The study revealed that smaller SWC values decreased the photosynthesis rate (Table 2). When the SWC was 20% at the PPFD of 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, as well as 20% and 30% at the PPFD of 1000  $\mu$ mol CO<sub>2</sub> per 1  $m^2 \cdot s^{-1}$ , the respiration process prevailed over the photosynthesis process. Therefore, the figures referring to photosynthesis had negative values. For the PPFD of 1000 µmol·m<sup>-2</sup>·s<sup>-1</sup> at the SWC ranging from 20% to 50% photosynthesis did not differ significantly. A considerable increase in photosynthesis occurred only for the SWC of 60%. When measurements were taken at the PPFD of 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, photosynthesis ranged from  $-0.51 \mu$ mol CO<sub>2</sub> per 1 m<sup>2</sup>·s<sup>-1</sup> at 20% SWC to 6.62 \mumol CO<sub>2</sub> per 1 m<sup>2</sup>·s<sup>-1</sup> at 60% SWC. Strong influence of drought stress on net photosynthesis in cauliflower seedlings was also confirmed by Wu et al. (2012). These authors found that 8 days of drought stress resulted in about 60% decrease in net photosynthesis. The response to water stress strictly depends on the plant species. Hassan (2006) observed that drought stress decreased the photosynthesis rate by as much as 60% in Triticum aestivum. Ahmed et al. (2002) in their study on mung bean discovered that after 8 days of drought stress the photosynthesis rate decreased by 75% in plants at the stage of vegetative growth and by 81% in those at the stage of generative growth. Galmés et al. (2007a) observed a 30% decrease in photosynthesis when the SWC decreased by 50% in Lysimachia minoricensis. A significant decrease in the photosynthesis rate under drought stress was also confirmed by Warren (2008) in his study on tomato and pea, which amounted to 65% and 47%, respectively.

Table 1. Amount of water lost from the substrate depending on the substrate water content (SWC) after 9 days of drought stress

Tabela 1. Ilość wody utraconej przez substrat w zależności od zawartości w nim wody (SWC) po 9 dniach stresu suszy

SWC (%)	Real substrate water content Rzeczywista zawartość wody w substracie (% FC)	Amount of evaporated water Ilość wyparowanej wody (l)
20	25.8	3.026 a
30	27.2	2.652 ab
40	40.0	2.820 ab
50	52.3	2.396 b
60	74.6	1.580 c

Mean values followed by the same letters do not differ significantly at P = 0.05.

Wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0,05.

Table 2. Effect of the substrate water content (SWC) on the photosynthetic rate (A) and transpiration rate (E) after 9 days of drought stress

Tabela 2. Wpływ zawartości wody w substracie (SWC) na tempo fotosyntezy (A) i tempo transpiracji (E) po 9 dniach stresu suszy

SWC (%)	(μmol CO <sub>2</sub> (μmol CO <sub>2</sub>	A per 1 $m^2 \cdot s^{-1}$ ) na 1 $m^2 \cdot s^{-1}$ )	l (mmol H2O (mmol H2O	$\frac{1}{1} per 1 m^{2} \cdot s^{-1} $ na 1 m <sup>2</sup> · s <sup>-1</sup> )
	PPFD 1000 μmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1700 μmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1000 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1700 μmol·m <sup>-2</sup> ·s <sup>-1</sup>
20	-0.38 b	-0.51 d	0.25 b	0.19 d
30	-0.20 b	2.81 c	0.26 b	0.65 c
40	0.61 b	4.66 b	0.33 b	1.13 b
50	0.57 b	5.24 ab	0.36 b	1.11 b
60	4.56 a	6.62 a	0.98 a	1.68 a

Mean values followed by the same letters do not differ significantly at P = 0.05, separately for each PPFD level.

Wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0.05, osobno dla każdego poziomu PPFD.

The first response of plants to drought stress is stomatal closure to limit water loss (Flexas and Medrano, 2002). In our study the transpiration rate (E) was influenced by the SWC (Table 2). It reached the highest values at 60% SWC at both PPFD levels. When the PPFD was 1700  $\mu$ mol·m<sup>-2·</sup>s<sup>-1</sup>, the transpiration rate at 20% SWC was almost 3.5 times lower than at 30% SWC and almost 9 times lower than at 60% SWC. Miyashita et al. (2005) in a study on kidney bean observed a significant decrease in transpiration rate after 2 days of drought. However, after 7 days the transpiration rate was almost zero.

According to Warren (2008), a drop in the photosynthetic rate was accompanied by a decrease in stomatal conductance ( $g_s$ ). In this way plants can limit the loss of water and improve the efficiency of its use (Chaves et al., 2009). Galmés et al. (2007b) claim that  $g_s$  is the basic stomatal factor inhibiting photosynthesis. Warren et al. (2004) noticed that under drought stress a 20-50% decrease in the photosynthetic rate in Douglas fir seedlings (*Pseudotsuga menziesii*) was accompanied by a 40-70% drop in  $g_s$ . Campos et al. (2014) examined A and  $g_s$  values in pepper after 4 and 9 days of drought stress. In both cases the parameters decreased considerably – photosynthesis by 65% and 11%, whereas stomatal conductance by 60% and 95%, respectively. Our study also confirmed the tendency of changes in stomatal conductance under drought stress. After 9 days of exposing cauliflower to drought stress the stomatal conductance rate decreased along with the SWC, both at the PPFD of 1000  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> and 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> (Table 3). However, there were no significant differences between the 20–50% SWC ranges.

The net photosynthetic rate is closely related to the electron transport rate (ETR) (Foyer et al., 1990). According to Flexas et al. (1999), the ETR value depended on drought stress and the PPFD value. When light intensity ranged from 1000 to 1200

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Table 3. Effect of the substrate water content (SWC) on the stomatal conductance  $(g_s)$  and electron transport rate (ETR) after 9 days of drought stress

Tabela 3. Wpływ zawartości wody w substracie (SWC) na przewodnictwo szparkowe (gs) i tempo transportu elektronów (ETR) po 9 dniach stresu suszy

SWC (%)	g <sub>s</sub> (mmol H <sub>2</sub> O per 1 m <sup>2</sup> ·s <sup>-1</sup> ) (mmol H <sub>2</sub> O na 1 m <sup>2</sup> ·s <sup>-1</sup> )		ETR (µmol·m <sup>-2</sup> ·s <sup>-1</sup> )	
	PPFD 1000 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1700 μmol·m <sup>-2</sup> ·s <sup>-1</sup>	$PPFD \ 1000 \ \mu mol \cdot m^{\text{-2}} \cdot s^{\text{-1}}$	PPFD 1700 µmol·m <sup>-2</sup> ·s <sup>-1</sup>
20	0.01 b	0.01 b	66 a	101 b
30	0.01 b	0.04 b	79 a	130 a
40	0.02 b	0.07 b	82 a	144 a
50	0.02 b	0.06 b	73 a	148 a
60	0.09 a	0.20 a	85 a	153 a

Mean values followed by the same letters do not differ significantly at P = 0.05, separately for each PPFD level.

Wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0.05, osobno dla każdego poziomu PPFD.

 $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, the ETR value decreased as drought stress increased, whereas at low light intensity (200  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>) the ETR had similar values regardless of drought stress. In our study the difference in the ETR values for 60% and 20% SWC was 22% at the PPFD of 1000  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> and 34% at the PPFD of 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> (Table 3). The mean value of A at the PPFD of 1700  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup> was 3 times higher than at the PPFD of 1000  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>, while the ETR was twice as high.

According to Campos et al. (2014), stomatal limitation of the photosynthesis process is crucial at the beginning of drought stress, while the nonstomatal mechanism appears under prolonged stress conditions. Lawlor and Tezara (2009) claim that after stomatal closure at first the  $CO_2$  content in leaves (C<sub>i</sub>) decreases along with increasing stress and then it grows under strong stress. A drop in C<sub>i</sub> shows dominance of the stomatal factor inhibiting photosynthesis and it does not depend on metabolic factors. At a certain stage of drought, the range of which is represented by the  $g_s$  value, the  $C_i$  value often increases. It shows prevalence of the nonstomatal factor as a photosynthesis inhibiting factor. Under strong drought stress metabolic changes occur and decrease the ribulose-1.5--bisphosphate (RuBP) content. This is the main factor inhibiting CO<sub>2</sub> photosynthesis (Flexas and Medrano, 2002). In this study there was a twofold difference in the  $CO_2$ content in substomatal cavities between plants grown at 60% and 20% SWC at the PPFD of 1700 µmol·m<sup>-2</sup>·s<sup>-1</sup> (Table 4). At the PPFD of 1000 µmol·m<sup>-2</sup>·s<sup>-1</sup> the difference in the  $CO_2$  content in substantial cavities at the extreme water content values (60% and 20%) was only 36%, but it was statistically significant. On the basis of the literature cited above we can assume that in our study the nonstomatal mechanism was the predominant factor inhibiting photosynthesis.

According to Efeoglu et al. (2009), drought stress decreases chlorophyll fluorescence. Zlatev and Yordanov (2004) carried out an experiment on three bean cultivars Table 4. Effect of the substrate water content (SWC) on the intercellular CO<sub>2</sub> concentration (C<sub>i</sub>) after 9 days of drought stress (ppm)

Tabela 4. Wpływ zawartości wody w substracie (SWC) na zawartość międzykomórkowego CO<sub>2</sub> (C<sub>i</sub>) po 9 dniach stresu suszy (ppm)

SWC	Ci		
(%)	PPFD 1000 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1700 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	
20	393 a	463 a	
30	319 ab	272 b	
40	290 ab	215 bc	
50	311 ab	187 c	
60	251 b	232 bc	

Mean values followed by the same letters do not differ significantly at P = 0.05, separately for each PPFD level.

Wartości średnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0.05, osobno dla każdego poziomu PPFD.

and observed small changes in  $F_v/F_m$  values under drought stress. However, there were considerable differences in the quantum yield of electron transport (Y). In our study the Y value decreased as drought stress increased, but the differences were not statistically significant. Additionally, there were slight differences in chlorophyll fluorescence (from 0.77 to 0.79), but there were considerable differences in photosynthesis both at the PPFD of 1000 µmol·m<sup>-2</sup>·s<sup>-1</sup> and 1700 µmol·m<sup>-2</sup>·s<sup>-1</sup> (Table 5). A similar tendency was noted by Miyashita et al. (2005), who used kidney bean in their research. They observed

Table 5. Effect of the substrate water content (SWC) on the chlorophyll fluorescence  $(F_\nu/F_m)$  after 9 days of drought stress

Tabela 5. Wpływ zawartości wody w substracie (SWC) na fluorescencję chlorofilu  $(F_v/F_m)$  po 9 dniach stresu suszy

SWC (%)	F <sub>v</sub> /F <sub>m</sub>	Yield, F <sub>vs</sub> /F <sub>ms</sub> Wydajność, F <sub>vs</sub> /F <sub>ms</sub>	
		PPFD 1000 µmol·m <sup>-2</sup> ·s <sup>-1</sup>	PPFD 1700 µmol·m <sup>-2</sup> ·s <sup>-1</sup>
20	0.77 a	0.16 a	0.14 a
30	0.78 a	0.19 a	0.18 a
40	0.79 a	0.20 a	0.20 a
50	0.79 a	0.17 a	0.21 a
60	0.78 a	0.20 a	0.21 a

Mean values in column followed by the same letters do not differ significantly at P = 0.05. Wartości średnie w kolumnie oznaczone tymi samymi literami nie różnią się istotnie przy P = 0.05. Krzesiński, W., Spiżewski, T., Kałużewicz, A., Frąszczak, B., Zaworska, A., Lisiecka, J. (2016). Cauliflower's response to drought stress. Nauka Przyr. Technol., 10, 4, #44. DOI: http://dx.doi.org/10.17306/J.NPT.2016.4.44

that a significant decrease in photosynthesis, transpiration and stomatal conductance occurred after only 2 days of drought stress, whereas a considerable decline in chlorophyll fluorescence was observed after 7 days of exposure to the stress. According to Galmés et al. (2007a), under drought stress, the light which is in excess of what can be used in photosynthesis increases, resulting in photoprotection and/or photoinhibition. When protective mechanisms occurred as a result of strong drought stress, a 70% decrease in photosynthesis was accompanied by merely a 5% decline in chlorophyll fluorescence ( $F_v/F_m$ ). In our study the difference in  $F_v/F_m$  values was small at the extreme SWC levels. However, there was a considerable difference in photosynthesis activity. Such great changes in A values, as compared to slight differences in  $F_v/F_m$ , can be explained by the occurrence of photoprotection in the cauliflower plants under study.

# Conclusions

The response of plants to drought stress showed a significant correlation with the SWC. At the SWC of 20%, the substrate moisture remained the lowest and as a result, the plants grown in this substrate absorbed the smallest amount of water. In this treatment cauliflower plants showed the lowest rates of net photosynthesis, transpiration and stomatal conductance, while the  $CO_2$  content in substomatal cavities and electron transport rate were the lowest. The chlorophyll fluorescence remained stable. The decrease in photosynthesis resulted from limited gas exchange through stomatal conductance. It caused a decrease in  $g_s$ , rise in  $C_i$  and limited electron transport rate.

We can conclude that lower SWC values resulted in a faster occurrence of shortage of water in the substrate. It caused the plants' stronger and quicker response to drought stress.

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## REAKCJA KALAFIORA NA STRES SUSZY

#### Streszczenie

**Wstęp.** Kalafior ma duże zapotrzebowanie na wodę ze względu na znaczną masę liści, dlatego też stres suszy jest jednym z głównych czynników wpływających na plonowanie tej rośliny. Celem wykonanych badań było określenie skutków stresu suszy na parametry fizjologiczne roślin kalafiora.

**Material i metody.** Kalafior odmiany 'Sevilla'  $F_1$  był uprawiany w pojemnikach, w podłożu o zróżnicowanej zawartości wody (20%, 30%, 40%, 50% i 60%). Wykonano następujące pomiary: intensywność fotosyntezy, transpiracja, fluorescencja chlorofilu, przewodnictwo szparkowe, zawartość międzykomórkowego CO<sub>2</sub> i dynamika transportu elektronów. Pomiary wykonano w fazie 5 liści po 9 dniach suszy.

**Wyniki.** Najmniejszą wilgotność stwierdzono w przypadku kombinacji o 20-procentowej zawartości wody w substracie w stosunku do polowej pojemności, w wyniku czego rośliny rosnące w tym podłożu pobrały najmniej wody. Badania wykazały, że niższe poziomy zawartości wody w podłożu powodowały szybsze występowanie stresu suszy. Przy 20-procentowej zawartości wody w substracie rośliny kalafiora charakteryzowały się mniejszą niż w pozostałych kombinacjach dynamiką fotosyntezy, słabszą transpiracją i gorszym przewodnictwem szparkowym, a zawartość CO<sub>2</sub> międzykomórkowego była najmniejsza oraz transport elektronów był najsłabszy. Nie stwierdzono wpływu zawartości wody w podłożu na fluorescencję chlorofilu.

Wnioski. Stwierdzono istotną zależność pomiędzy zawartością wody w substracie a reakcją roślin na stres suszy: wraz z większą zawartością wody opóźniało się występowanie stresu suszy. Z drugiej strony niski poziom zawartości wody w substracie powodował szybszą reakcję rośliny na stres suszy.

Slowa kluczowe: Brassica oleracea L. var. botrytis subvar. cauliflora DC., transpiracja, fotosynteza, fluorescencja chlorofilu, przewodnictwo szparkowe, transport elektronów

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