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Resistance of *Sarepta mustard* to a decrease in ambient temperature during vegetation

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Abstract. *Brassica juncea* (L.) Czern. is an important edible oilseed crop. This species is currently well studied from the point of view of its use in the phytoremediation of contaminated soils. There is practically no information about the resistance of Sarepta mustard to low temperatures. However, they are important for identifying the possibility of growing this species in northern conditions. In this regard, the effect of low temperature (4° C) on the *B. juncea* var. Nika growth, photosynthesis and water exchange was studied. An exposure to 4 °C inhibit the plants' growth, but the photosynthesis rate does not exchange. At the same time, under the hypothermia, the chlorophyll *a* content and the chlorophylls *a*/*b* ratio increase. The carotenoids content and the size of stomatal aperture remained at the optimal temperature conditions level, and the F*v*/F*m* increased, indicating the efficient operation of photosystem II. Being exposed to low temperatures, the transpiration rate slowed down, which ensured the maintenance of the necessary tissue hydration and increased the ability to efficiently utilize water in the photosynthesis (WUE). A conclusion was made about the resistance of *B. juncea* var. Nika to low temperature (4° C) and about the possibility of growing this species in northern regions, including the phytoremediation of polluted areas.

1 Introduction

The *Brassica juncea* (L.) Czern. (Sarepta mustard) family (Brassicaceae) is well known as an oilseed, forage, medicinal and sideral crop [1, 2]. Mustard oil, due to its high content of essential amino acids, is used in the food industry, including canning, confectionery, baking industries. It is used for technical purposes in soap making, textile industry, perfumery and medicine [3]. Currently, Sarepta mustard is one of the most valuable crops, which is recommended for farming in different regions. This is due to its high resistance to a number of unfavourable environmental factors, such as, drought, salinization, deficiency of mineral nutrition elements in the soil [2]. In addition, Sarepta mustard is able to grow in soils with high content of heavy metals [4, 5]. The levels of metal accumulation by *B. juncea* do not qualify it as a "hyperaccumulator". However, the ability of this species to accumulate in large amounts a wide range of heavy metals, including cadmium, copper, lead, chromium and zinc, in addition to its rapid growth and large biomass, makes Sarepta mustard promising for phytoremediation of HM-contaminated areas [6]. Consequently, *B. juncea* is being actively studied in terms of its metal tolerance and phytoremediation potential.

In northern regions, the successful use of plants for soil purification from heavy metals is difficult due to frequent temperature drops to low values during vegetation [7, 8]. Therefore, knowledge of plants cold tolerance is needed when selecting sites for soils phytoremediation under these conditions. For *B. juncea*, there is no information on the ability of this species to adapt to lower temperatures in the literature known to us. Although other members of this family, such as *Brassica napus* [9] and *Brassica oleracea* [10], have been shown to successfully adapt to low-temperature conditions.

The aim of this work was to study the tolerance of Sarepta mustard to a short-term temperature decrease based on a growth parameters, photosynthesis activity and water metabolism.

2 Material and methods

2.1 Plant material and growth conditions

Seeds of Sarepta mustard (*Brassica juncea* (L.) Czern.) of the Nika var. were germinated in distilled water for 3 days. The seeds were provided by the Department of Genetic Resources of Oilseed and Fiber Crops at the Federal Research Center "N.I. Vavilov All-Russian Institute of Plant Genetic Resources". The seedlings were transferred in hydroponic conditions and grown in a Hoagland-Arnon nutrient solution under light at a temperature of 22º C and the relative air humidity of 60–70%. PAR was 200 μmol $(m^2 \cdot s)^{-1}$, and there was a 14-hour photoperiod. After 14 days (at the 1st true leaf stage), one group of plants was kept at 22º C (control), while the other group was placed in a cold chamber at 4º C for 7 days.

After the experiment, cold tolerance was assessed based on growth parameters (shoot height, shoot dry biomass, area of the 1st true leaf), parameters of photosynthetic apparatus activity (the content of photosynthetic pigments, F*v*/F*m*, stomatal conductance, photosynthesis rate) and water exchange (the

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transpiration rate, the shoot water content and water use efficiency) of the plants.

2.2 Biometric measurements

For ten plants of each experimental variant, the shoot length, dry biomass, and the first true leaf area were measured. The dry mass of the shoots was measured after drying at 105°C to a constant weight. The leaf area was determined using the AreaS 2.1 software.

2.3 Photosynthetic pigments content and the activity of PS II measurements

Chlorophyll a, chlorophyll b and carotenoids were measured and analysed spectrophotometrically using the SF-2000 spectrophotometer (Spectrum, Moscow, Russia) and calculated using the established formulas [11]. For the extraction of pigments the 98% (v/v) acetone solution was used. The percentage of chlorophylls in light-harvesting complex II (LHCII) was calculated according to [12]. To measure the pigments content, three mixed samples of five leaves were taken. The analytical replication was threefold. The activity of PS II was assessed by the F*v*/F*m* parameter, which characterizes the potential quantum yield of photochemical activity, using a MINI-PAM pulse-modulated fluorometer (Walz, Germany) after leaves dark adaptation during 20 minutes. For measurements of the F*v*/F*m* parameter ten biological replicates were performed.

2.4 Stomatal conductance, a photosynthesis rate, and transpiration rate measurements

Stomatal conductance, a photosynthesis rate, and a transpiration rate were measured using a portable system for CO_2 gas exchange and water vapour analysis (HCM-1000, Germany). For measurements of these parameters ten biological replicates were performed.

2.5 Water exchange parameters measurement

The water use efficiency (WUE) was calculated as the ratio of the apparent photosynthesis rate to transpiration. The water content (WC) was determined according to Turner [13]. The leaves were weighed (fresh weight, FW); then they were dried in an oven at 80 °C for 24 h and weighed again (DW). The water content (WC) was estimated in percentage (%) using the following formula: WC = ([FW – DW]/FW)*100, where FW is the fresh weight and DW is the dry weight. For measurements of these parameters ten biological replicates were performed.

2.6 Statistical analysis

All experiments were performed two times with three replicates for each variant. The experimental data were expressed as means ± standard errors (SE). The data were analysed by one-way analysis of variance (ANOVA) using Microsoft Excel 2010. Student's *t*-test was applied to compare statistical significance at the level of $p < 0.05$.

3 Results and discussion

According to literature data, the optimal temperature conditions for successful growth of Sarepta mustard are within 12-25 °C [1], so a temperature drop to 4 °C, which is often encountered in northern latitudes during vegetation, is stressful for plants of this species. It is known that the process of cold adaptation is an energy-intensive process that requires energy investments from the plant organism [14]. Therefore, successful plants adaptation to cold is determined by the ability to rapidly and co-ordinately change the intensity of the main physiological processes, primarily growth and photosynthesis, associated with the formation, redistribution, and utilization of plastic substances and energy. In particular, one of the characteristic responses of plants to the low temperature is growth inhibition, which allows them to save energy reserves, directing them to the maintenance of energy-consuming processes, including photosynthesis [15, 16]. In this experiment, it was found that after 7-day exposure of Sarepta mustard to a 4 °C shoot growth retards. Therefore, the shoot height and its dry biomass in the experimental condition were 36 and 60 % lower than those in the control, respectively (table 1), and the area of leaf formed during exposure was 62 % lower than that in the control (table 1, figure 1).

Table 1. The effect of low temperature (4 °C) on shoot growth parameters of *Brassica juncea* var. Nika

Parameter	Temperature	
		4 °C
Shoot height, cm	15.57±0.29 a	10.03±0.18 b
Shoot dry biomass, g	0.055±0.004 a	0.022±0.001 b
Leaf area, cm^2	4.36±0.33 a	1.66±0.13 b

Different letters denote significant differences between control and experimental variants at $p < 0.05$.

A B

Fig. 1. The general overview of the effect of optimal (22 °C) (A) or low (4 °C) (B) temperature on the first true leaf formation of the *Brassica juncea* var. Nika

The study also revealed that under the hypothermia condition the photosynthesis rate was maintained at the control level (figure 2a). It is known that photosynthesis is the main target for low temperatures, so the ability of plants to maintain its intensity at a high level under low temperatures indicates their cold tolerance [17, 18].

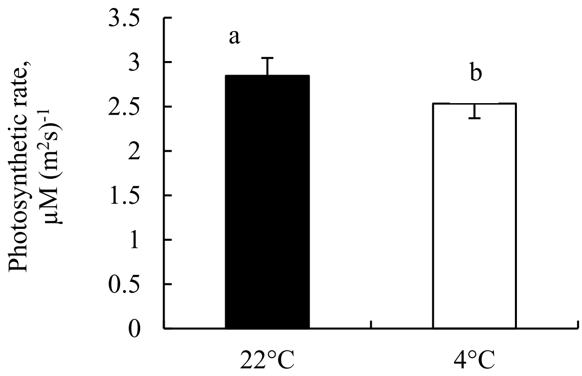

Fig. 2. The effect of low temperature (4 °C) on the photosynthetic rate of *Brassica juncea* var. Nika

A number of adaptive changes in photosynthetic activity parameters contributed to the preservation of the high photosynthesis level. In particular, the content of chlorophyll *a*, as well as the ratio of chlorophyll *a*/*b*, increased (compared to the control) under the cold due to the absence of changes in the chlorophyll *b* content (table 2). However, the content of chlorophylls in the LHC of the experimental plants was significantly lower than that of the control (table 2).

Table 2. The effect of low temperature (4 °C) on pigments content and F*v*/F*m* in *Brassica juncea* var. Nika

Parameter	Temperature	
	22 °C	4 °C
Chlorophyll *a* content, mg g^{-1} FW	0.45±0.014a	0.47±0.006b
Chlorophyll *b* content, mg g^{-1} FW	0.21±0.009a	0.20±0.005a
Carotenoids content, mg g^{-1} FW	0.13±0.004a	0.13±0.003a
Chlorophylls ratio (*a*/*b*)	2.17±0.049b	2.38±0.047a
LHC chlorophylls content, %	69.65±1.12a	65.35±0.89 b
F*v*/F*m*	0.760±0.002 b	0.800±0.004 a

Different letters denote significant differences between control and experimental variants at $p < 0.05$.

The increase in the chlorophylls ratio is known to be an indicator of stress adaptation of the photosynthetic apparatus and is aimed at enhancing light absorption while decreasing the proportion of chlorophylls in LHCs of photosystems [19, 20]. A similar effect was also found in *B. oleracea* at 8 °C [7] and *Triticum aestivum* at 4 °C [18]. In addition, a high level of carotenoids content was maintained in Sarepta mustard under cold exposure. Since carotenoids perform a number of important functions in the photosynthesis process, including antenna functions, acting as additional "light collectors", as well as being active antioxidants, these pigments are considered as one of the factors providing plant resistance to unfavourable temperature effects [21].

Photosystem II plays a central role in the generation and regulation of electron transport in chloroplasts. Therefore, the indicators reflecting the efficiency of its work are important for the assessment of the activity of photosynthetic apparatus [8]. A number of studies have shown that low temperatures negatively influence the functioning of photosystems, which, for example, was found in rice under 12 °C [22], in a number cultivars of *B. napus* under 10 °C [8], and in *Glycine max* under 6 °C [23]. Preservation at 4 °C in experimental mustard plants of the F*v*/F*m* value, characterizing the quantum efficiency of photosystem II, at the control level, which was found in the experiment, indicates the absence of disturbances in its functioning [24].

As a rule, in plants, in response to cold exposure, the level of endogenous ABA increases in the closing cells of stomata, which leads to their partial closure. The resulting decrease in stomatal conductance and the transpiration rate is a defence-adaptive reaction that allows plants to avoid significant dehydration under these conditions [25, 26]. No such effect was observed in our studies. The stomatal conductance under 4 °C did not differ from that under 22 °C, indicating the absence of severe stress in plants (figure 3). The preservation of stomatal conductance under hypothermia at the control level was probably one of the reasons for maintaining the necessary photosynthetic rate under these conditions.

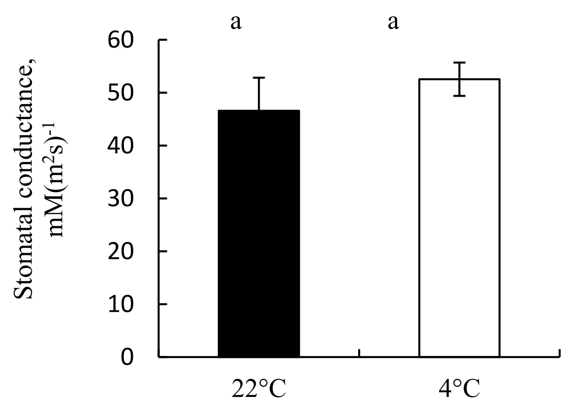

Fig. 3. The effect of low temperature (4 °C) on stomatal conductance of *Brassica juncea* var. Nika

At the same time, the rate of transpiration under cold exposure decreased by 21% compared to the control (table 3). This contributed to the preservation of water content in tissues. It is known that the ability of plants to maintain the optimal level of water balance under low-temperature exposure is characteristic of cold-resistant plants. In addition to decreased the transpiration rate, the cells of these plants have the ability to retain water, for example, by increasing the sugars synthesis [18]. They are also characterised by a high level of osmoregulation [27]. In addition, in cold-tolerant plants, the hydraulic conductivity of roots increases, resulting in increased water flow into shoots [28, 29].

Table 3. The effect of low temperature on water metabolism parameters of *Brassica juncea* var. Nika

Parameter	Temperature	
	22°C	4°C
Transpiration rate, mmol H_2O $(m^2s)^{-1}$	0.61±0.09 a	0.48±0.08 b
Water content, %	94.0±0.1 a	95.0±0.1 a
WUE, µmol CO_2 /mmol H_2O	3.43±0.5 b	5.42±0.3 a

Different letters denote significant differences between control and experimental variants at $p < 0.05$.

It is also important to note that WUE increased in mustard when exposed to 4 °C. It is believed that the ability of plants to successfully adapt to stress conditions is provided by their ability to efficiently utilize water in the photosynthesis. This allows the plant organism to allocate resources qualitatively and direct them to the synthesis of plastic substances used to maintain growth and biomass accumulation [30].

4 Conclusions

In conclusion it is necessary to note that *B. juncea* var. Nika successfully adapts to the short-term (within 7 days) decrease of temperature (4° C) during vegetation. This is facilitated by a number of adaptation mechanisms. The first is enhancement under hypothermia of chlorophyll *a* synthesis and an increase in the chlorophylls *a*/*b* ratio. The second is the ability to maintain the carotenoid content at the control level, which contributes to light absorption when the amount of LHC chlorophyll decreases. The third is maintenance of the stomatal aperture size at the control level, providing the necessary rate of photosynthesis. The adaptation reaction was also inhibition of shoot growth under cold conditions, aimed at redistribution of energy resources in favour of photosynthesis, as well as slowing down the transpiration intensity, allowing maintaining the necessary level of the water content. The results allow concluding about the possibility of growing *B. juncea* var. Nika in northern regions, including for phytoremediation of polluted areas.

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6 Declaration of conflict of interest

The authors declare no conflict of interest.

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