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INFLUENCE OF INCREASED CO_2 CONCENTRATIONS ON FROST RESISTANCE OF WINTER CEREALS

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A b s t r a c t. Tests for resistance to frost were conducted by means of luminescence and conductometric methods for seedlings both unhardened and hardened within 14 days at various CO_2 concentrations (400, 800, 1200 and 1600 ppm). The tests proved that photosynthetic apparatus and cell membranes of seedlings raised at various CO_2 concentration are differential in frost resistance.

K e y w o r d s: carbon dioxide, frost resistance, winter cereals

INTRODUCTION

The results of studies by various authors do not provide a clear-cut answer to the problem of plant response to increased concentration of CO_2 in the atmospheric air [1,7,8]. It appears to be of interest whether increased concentration of CO_2 modifies the tolerance of the photosynthetic apparatus and the resistance of cell membranes and whole plants to stress-inducing factors, e.g. low temperature. Increased concentration of carbon dioxide usually stimulates the growth and development of winter plants. This may lead to their freezing, due to winter period occurring at the wrong stage of their development. Moreover, one can expect that the process of frost hardening of plants will also be subject to modification. The authors of this work have undertaken an attempt at solving the problem.

MATERIAL AND METHODS

From the list of crop plants published by the national research centre for agricultural crops (Centralny Ośrodek Badania Odmian Roślin Uprawnych) two cultivars each of winter cereals were selected, with varied frost resistance characteristics.

- Barley – *Sigra* and *Tramp*;
- Wheat – *Almari* and *Roma*;
- Triticale – *Alzo* and *Prado*;
- Rye – *Amilo* and *Wibro*

Aspects taken into consideration in the selection of cultivars included their being grown in the region of north-western Poland and their frost resistance characteristics, to obtain a better image of seedling hardening at various concentrations of CO_2.

Preparation of material for frost resistance estimation

Kernels of the particular cereal species and cultivars were placed on sheets of blotting paper which were then rolled into tubes and placed vertically in crystallizers. The rolled tubes prepared in this manner were flooded with diluted Hoaglanda medium (1:1). Until the beginning of hardening, i.e. for 14 days, the level of the solution was maintained at 1 cm above the crystallizer bottom. During the hardening period, the crystallizers were flooded daily with the diluted medium for 15 minutes, then the solution was drained out.

The plants grew under conditions as follows: PPFD irradiation of 200 μmol $m^{-2}s^{-1}$ PAR, photoperiod 12h/12h day/night, temperature 10°C, at 4 levels of CO_2 concentration in the w atmosphere: 400 ppm, 800 ppm, 1200 ppm and 1600 ppm. The concentration of CO_2 was controlled by means of special systems coupled to CO_2 sensors (AirTech 2600). After two weeks, the hardening of seedlings was begun, gradually reducing temperature to +2°C, and PPFD irradiation to 50 μmol $m^{-2}s^{-1}$ with a photoperiod of 8h/16h day/night.

Conductometric method

The conductmetric method developed by Dexter *et al*. [5] is based on the assumption that damage to plants caused by low temperature results from the loss of properties of semi-permeable cell membranes. Depending on the degree to which low temperature caused changes in the permeability of the membranes or damaged the cell membranes, more or less ions will be penetrating to water. Therefore, we obtain a solution and, measuring its electric conductivity, we can conclude the condition of the cell membranes – the higher the conductivity the more damaged the cell membranes. So, measurement of the values of electric conductivity can be used to compare the sensitivity of the plant objects studied to low temperature [9]. However, there is a serious shortcoming to the method, as the relation is not necessarily linear, and the objects compared have to be of the same size, otherwise it is necessary to convert the values of electric conductivity per unit of the object volume or mass.

The conductometric tests were performed in accordance with a modified procedure as described by Brzóstowicz and Prokowski [3]. Above-ground parts of plants were used for the measurements.

Next, the index of injury was calculated according to the Flint formula [6].

The calculated index of injury expresses the percentage value of damage to seedlings with relation to the maximum damage level than may occur with „total" destruction of the seedlings be low temperature.

Due to the insufficient output of the chambers used for the freezing, we were unable to obtain temperature reduction at the required rate below the level of -22°C. In certain variants that temperature proved to be too high and there was no possibility of obtaining 50% levels of damage, most frequently used in estimations of frost resistance, as some of the cereals tested were too resistant to frost. For this reason, to be able to compare the results obtained by means of the conductometric method for all the seedlings tested, it was decided to determine the level of freezing temperature at which 35% level of injury was recorded – „t_{35}".

The values of the t_{35} parameter, presented in the graphs of the relation of the index of injury (I_d) to the freezing temperature, represent the temperature at which 35% electrolyte seepage occurs, which may be adopted as 35% damage to plant tissue (cell walls and membranes).

The level of error did not exceed ±1.5°C taking into account the error of determination I_d calculated with the method of total differential.

Delayed luminescence method

The measurements were made with the help of a test set built by Brzóstowicz and subsequently modified at the Department of Physics, Academy of Agriculture in Szczecin [2,3].

For the tests fragments of first leaves were taken, with a length of 2 cm, cut from tip section of the leaves. To ensure uniform test conditions, prior to measurements the samples were placed for 30 minutes in closed and moist Petri dishes at a temperature +5°C and with photon flux density of PPFD 200 µmol $m^{-2}s^{-1}$. Then the samples were transferred to the measurement chamber and the measurements were taken.

The delayed luminescence (IOL) was excited with white light for 1 s and recorded for 1 s after 1 s from the moment of switching off the excitation lamp. At the same time the temperature of the samples tested was brought down from 0°C to –20°C at the rate of 1°C per minute.

The comparison of crop plant species or cultivars in terms of their frost resistance is based on specific parameters calculated from the OL thermogram obtained in the manner described above. The parameters are:

t_m – temperature at which the maximum intensity (I_{max}) of delayed luminescence occurred; its value indicates the temperature of electron transport blocking in photosystem II between electron carriers Q_a and Q_b;

W_w – relative increase in delayed luminescence intensity during the lowering of temperature;

$$W_w = \frac{I_{max} - I_{min}}{I_{min}}$$

where: I_{max} – maximum value of delayed luminescence intensity; I_{min} – minimum value of delayed luminescence intensity during the lowering of temperature.

The values of t_m and W_w may be used for the comparison of plants in terms of their current "resistance of photosynthetic apparatus" to low temperature. Lower temperature t_m and higher value of W_w under steady conditions of measurement indicate higher initial resistance of reactions in photosystem II to low temperatures, and that indicates greater frost resistance of the plant [3].

RESULTS AND DISCUSSION

The performed analysis of cereals sensitivity to low temperatures revealed a differentiation related to the concentration of CO_2, plant species, and even cultivar (Tab. 1). Moreover, in some of the variants of the experiment, differentiation was observed in the sensitivity to low temperature of cell membranes, tylacoidal membranes, or electron transport in photosystem II.

Table 1. CO_2 concentration at which frost resistance parameters testified to highest resistance of hardened seedlings

Species	Cultivar	W_w		t_m (°C)		t_{35} (°C)	
		nh	h	nh	h	nh	h
Barley	Sigra	d.i.	d.i.	d.i.	800	400	400
	Tramp	d.i.	d.i.	d.i.	r.n.	400	400
Wheat	Almari	800	d.i.	d.i.	1600	d.i.	400
	Roma	d.i.	d.i.	d.i.	1600	1200	1600
Triticale	Alzo	400	d.i.	400	1600	1200	1200
	Prado	400	400	d.i.	d.i.	1600	1600
Rye	Amilo	d.i.	d.i.	d.i.	d.i.	800	800
	Wibro	d.i.	d.i.	d.i.	d.i.	d.i.	1200

d.i. – differences insignificant at $\alpha = 0.05$.

Also the effect of frost hardening under conditions of increase concentration of CO_2 was highly differentiated depending on the variant of the experiment (Tab. 1).

In the case of the barley cultivars, the application of the luminescence method did not reveal any effect of CO_2 on the sensitivity of electron transport in photosystem II to low temperatures. On the other hand, the resistance of cell membranes of barley seedlings, determined by means of the conductometric method, was the highest at the control concentration of carbon dioxide. These effects were observed both for hardened and non-hardened plants.

Different results were obtained for wheat. For both cultivars the luminescence indexes obtained (W_w, t_m) indicate that the higher the concentration of CO_2 the lower the sensitivity of the photosynthetic apparatus to low temperatures. On the other hand, the resistance of cell membranes determined with the conductometric method was the highest at the control concentration of CO_2 in the case of the *Almari* cultivar, and for the frost resistant *Roma* at the highest CO_2 concentration applied. Therefore, differing results were obtained for the *Roma* wheat cultivar which, according to the catalogue of cultivars, is more frost resistant than *Almari*. In the case of the *Roma* cultivar, all the indexes determined (W_w, t_m, t_{35}) suggest an increase in the resistance of seedlings with increasing concentration of CO_2 in the atmosphere.

Also different were the results obtained for triticale. Here the tylacoidal membranes were the most resistant at the control concentration of CO_2. Electron transport in PS II of non-hardened seedlings of triticale was the least sensitive to low temperatures at the control concentration of carbon dioxide, while that of hardened seedlings – at the concentration of 1600 ppm CO_2. Different still was the resistance of triticale seedlings cell membranes. Both non-hardened and hardened seedlings of the *Alzo* cultivar had the highest frost resistance of cell membranes at the concentration of 1200 ppm, and those of the *Prado* cultivar at the highest CO_2 concentration applied.

All the indexes determined for rye (W_w, t_m, t_{35}) indicated that the various concentrations of CO_2 have no statistically significant effect on the sensitivity of seedlings to low temperatures. With detailed analysis, however, one can notice a tendency for the seedlings to become somewhat more resistant at the concentration of 800 ppm CO_2.

Recapitulating the results of the study was can conclude that:

– intensive development of barley seedlings under the effect of increased concentration of CO_2 did not significantly alter the sensitivity of electron transport in photosystem II to low temperature, but reduced the resistance of cell membranes in hardened plants, especially in the case of the cultivar with higher frost resistance;

– increased concentration of CO_2 had a weak effect on the biometric features of wheat seedlings, but increased the resistance of electron transport in photosystem II to low temperatures, and in the case of the more frost resistant cultivar Roma there was an additional increase in the resistance of cell membranes (reduced permeability) to low temperature;
– the most intensive growth of triticale seedlings and improved resistance to low temperature were observed at the triple concentration of CO_2;
– in the case of rye both the most intensive growth and the highest level of frost resistance occurred at the double concentration of CO_2.

The results presented and discussed herein concern seedlings taken for tests at the same phase of development. The concerns of certain researchers [1] that intensive development of plants at increased concentrations of CO_2 may reduce their resistance to low temperature did not find any specific confirmation in these experiment. Perhaps with an experiment of longer duration, if the plants became more differentiated in terms of biometric features and phase of development, the effect of increased concentration of CO_2 might be revealed in reduced frost resistance and absence of effective frost hardening.

CONCLUSIONS

1. Increased concentration of CO_2 has a significant effect on frost resistance of seedlings of wheat and triticale.

2. Increased concentration of CO_2 does not significantly affect frost resistance of seedlings of barley and rye.

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WPŁYW PODWYŻSZONEGO STĘŻENIA CO_2 NA MROZOODPORNOŚĆ ZBÓŻ OZIMYCH

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Streszczenie. W pracy przedstawiono wyniki dla wybranych odmian czterech gatunków zbóż ozimych pod względem ich mrozoodporności przy różnych stężeniach CO_2 (400, 800, 1200 i 1600 ppm CO_2). Wyniki obejmują pomiary przeprowadzone dwoma metodami: metodą konduktometryczną i metodą opóźnionej luminescencji.

Słowa kluczowe: CO_2, mrozoodporność, zboża ozime