УДК 633.11.324:581.036.5

ENZIMATIC DIAGNOSIS OF FROST RESISTENS IN WINTER WHEAT

Statsenko A.P., Blinokhvatov A.A.

We studied the variability of hydrolytic activity β-fructofuranosidase enzyme in the seedlings of different frost resistance of winter wheat varieties in the process of cold hardening and freezing. The close linkage between thermal environment, the activity of the enzyme and frost. To estimate the frost resistance of 15 winter wheat varieties of different ecological zones, resulting in on the basis of the indices of resistance divided into 3 groups of resistance: frost resistant index resistances 3.3-4.2 (Photinia, Claudia, Mironovskaya 808, Turquoise, bezenchukskaja 380); srednevozrastnoe – index of resistance of 1.9-2.3 (Cinderella, brunette, Erythrospermum 2/01-3-04, Orenburgskaya 105, Kinel 5); slavomacedonia – index of resistance of 1.0-1.4 (Basalt, Lutesens 2/01-17-04, bezenchukskaja 616, Erythrospermum 12/00-16-04, Santa). Hydrolytic activity of the enzyme is proposed to use as a performance indicator of frost resistance of new winter wheat varieties. The degree of variability of the hydrolytic activity of the enzyme β-fructofuranosidase in seedlings of winter wheat (index of resistance) allows objective evaluation of frost resistance of varieties and breeding material. Using this method has allowed to establish that in Penza research Institute of agriculture of new varieties of winter wheat Claudius and Photinia are highly resistant to low temperatures.The resistance index that expresses the degree of activation of the enzyme β-fructofuranosidase the cooling of the sprouts, it is an objective measure of frost resistance of winter wheat varieties, as evidenced by the survival in the freezing cold room.

Keywords: *winter wheat, freezing, frost resistance, β-fruktofuranozidaza, index of resistance, group resistance, survival, low-temperature stress.*

Introduction

In the forest-steppe zone of the Volga region, the main grain food crop is winter wheat. Under her crops in the region employs more than 2 million hectares, and the yield is about 2 t/ha. Under good weather conditions, this culture gives high yields of grain, as compared to spring, with longer periods of growth and development, uses the autumn, winter and early spring moisture reserves in the soil and time to establish the crop before the onset of summer drought. But in some adverse weather conditions in the winter period winter wheat is losing its advantages, because the crops die while overwintering.

One of the main causes of death of the winter wheat crop is in the region of varieties with low level of frost resistance which, as a rule, determines the stability of yield (12).

Pernicious influence of winter frosts on the crops is greatly enhanced with the denial of basic technological methods of cultivation, which exacerbates the decrease of frost resistance and leads to loss of crops.

In this regard, the study of the formation mechanism of frost resistance of winter crops and development of methods for its evaluation, undoubtedly, is relevant and promising.

In modern scientific literature has accumulated information about the role of enzymes of carbon metabolism in the formation of frost resistance of winter crops (2-4, 8).

Of special interest in connection with the reaction of infected plants in lowering the temperature causes a variation of the hydrolytic enzyme β-fructofuranosidase. This enzyme belongs to the class of hydrolases, which are able to accelerate the cleavage and synthesis of various organic compounds. In plants experiencing creatress, this enzyme splits sucrose into glucose and fructose. These carbohydrates, in particular the last two, able in the period of care of plants in winter to accumulate in the vegetative organs of winter crops and to perform cryoprotective function.

Researchers have demonstrated a close relationship between frost resistance of winter wheat, water regime, and the activity of β-fructofuranosidase (1, 2, 6). With that, activation of enzymes from cold-resistant wheat forms in the process of hardening is associated with more intensive dehydration of the cells. Increase of frost resistance in this case is linked to water deficiency in plant tissues.

Modern research shows that the metabolic processes of winter crops of various varieties in terms of creosote is genetically determined and therefore specific. This is true for the enzyme systems in General (8) and cleantime carbohydrate metabolism β-fructofuranosidase, in particular (5,9-11).

In this regard, we attempted to explore the use of indicator hydrolytic activity of this enzyme in the diagnosis of frost resistance of different varieties of winter wheat.

Research methodology

The object of research included 15 varieties of winter wheat, representing different ecological zones: Fotinia, Claudia, Mironovskaya 808, Turquoise, bezenchukskaja 380, Cinderella, darkie, Erythrospermum 2/01-3-04, Orenburgskaya 105, Kinel 5, Basalt, Lutesens 2/01-17-04, bezenchukskaja 616, Erythrospermum 12/00-16-04.

Temistocle plant seedlings grown in a growth chamber, were subjected to stepwise cooling in the refrigerating chamber in the following temperature regime: 1st day – 10-12 °C; 2 s – 0 - minus 2 °C; 3 -5 -7 °C; subsequent three days the temperature was -10 to - 12 °C.

In leaves of seedlings was determined by hydrolytic activity of the enzyme β-fructofuranosidase before and after cooling.

For this two-gram sample of leaves homogenized in 10 ml of distilled water until a homogeneous mass.

The homogenate was transferred into a volumetric flask of 50 ml, adjusted with distilled water to the mark and thoroughly mixed. After agitation of the slurry is selected two samples of 10 ml and transferred into volumetric flasks of 100 ml.

One flask (control) was heated to 100 °C and with the aim of inactivation of the enzyme was boiled for 2-3 minutes and then the suspension was cooled in an ice bath. After that in control and experimental flasks were added 5 playstatio buffer solution having a pH 5.0, 10 ml. of a 5% freshly prepared sucrose solution and 4 drops of toluene. The flask was then placed for 2 hours in a thermostat at 40 °C, after which was added 2.5 ml of 1N NaOH solution, 1 drop of phenolphthalein and dropwise 10% solution of zinc sulphate to a bleaching of the indicator. The volume of the solution was adjusted with distilled water to the mark, intensively stirred and filtered. In the obtained filtrate were determined the content of reduced sugars. To do this in a test tube with a diameter of 30 mm was added 5 ml. of the filtrate, the volume was adjusted with water to 10 ml, then was added 10 ml of a copper-alkaline solution, intensively stirred and placed for 15 minutes in a boiling water bath. The reaction was limited by immersion of the tubes in running tap water. To the cooled solution gradually was added 5 ml mixture of equal volumes of oxalic acid and sulfuric acid. When cuprous oxide is dissolved, the remaining iodine was titrated with 0.001 N sodium thiosulfate solution in the presence of 0.5 ml. 0.A 5% starch solution.

The activity of β-fructofuranosidase was determined for 1 h in 1 g of the test sample (7).

Based on the obtained results was calculated the index of resistance of plants which Express the relation of hydrolytic enzyme activity after stepwise cooling to the original. Then allocate 3 groups of resistance: frost resistant (resistance index of 3.00 and above); middle-aged (1.51–3.00) and submonotone (1.50 and below).

The results of the research

Analysis of the results of the experiment showed that the source of hydrothermal activity of the enzyme β-fructofuranosidase in the seedlings of all tested

Table 1 - Varietal characteristics hydrolytic activity of β-fructofuranosidase and frost resistance of winter wheat

A variety of wheat	The activity of the enzyme, mg/g/hour		The index of resistance	Group resilience (survival, %)
	To cool	After cooling		
Klavdiya	4.7	18.8	4.0	frost-resistant (92)
Fotinija	5.1	21.4	4.2	frost-resistant (96)
Mirinovskaya 808	4.9	16.2	3.3	frost-resistant (79)
Biruza	5.4	20.5	3.8	frost-resistant (87)
Bezenchukskaya 380	5.2	18.7	3.6	frost-resistant (80)
Zolushka	5.8	16.8	2.9	medium frost (76)
Smunglyanka	4.7	11.3	2.4	medium frost (67)
Erythrospermum 2/01-3-04	6.2	16.7	2.7	medium frost (72)
Orengburskaya 105	6.0	11.4	1.9	medium frost (63)
Kinelskaya 5	5.9	15.3	2.6	medium frost (70)
Bazalt	6.1	8.5	1.4	weakly frost-resistant (49)
Lutenstens 2/01-17-04	4.9	5.9	1.2	weakly frost-resistant (43)
Bezenchukskaya 616	5.3	6.9	1.3	weakly frost-resistant (45)
Erythrospermum 12/00-16 -04	5.8	5.8	1.0	weakly frost-resistant (42)
Santa	6.2	6.8	1.1	weakly frost-resistant (40)
NSR 095	0.3	0.7	0.2	1.8

varieties was relatively low, and varietal specificity of the studied biochemical basis was not revealed (table.1).

The cooling and subsequent freezing of seedlings contributed to the change in hydrolytic activity of the enzyme in the leaves of tested varieties. The most significant activity of the enzyme was recorded in the cultivars of winter wheat Claudius, Photinia, Mironovskaya 808, Turquoise and bezenchukskaja 380. This allowed us to include named varieties, hardy to the group that confirmed a high percentage of survival of seedlings after freezing in the cold room at -16...-18 °C. Less significant variation of enzyme activity in conditions of low temperature stress were different varieties of winter wheat Cinderella, brunette, Erythrospermum 2/01-3-04 Orenburg 105 and Kinelsky 5. This indicates a lower degree of readiness of plants to withstand low temperatures, in connection with what they referred to the group srednemnogoletnih that confirmed a lower percentage of survival after freezing. The weak response of plants to low temperature stress, judging by the slight variability of the enzyme, manifested in the varieties of winter wheat: Basalt, Lutescens 2/01-17-04, bezenchukskaja 616, Erythrospermum 12/00-16-04 and Santa, where the index of resistance ranged from 1.0 to 1.4, and the survival of shoots when freezing was no more than 49%. It allowed to consider the brand name to the group of weakly frost-resistant.

Digital statistical processing of experimental data allowed to conclude about the presence of significant differences among the studied cultivars of winter wheat in response to low temperature stress.

Our research has shown that the degree of variability of the hydrolytic activity of the enzyme β-fructofuranosidase in seedlings of winter wheat can be successfully used in breeding practice as one of the objective criteria of frost resistance of varieties and breeding material. So in Penza research Institute of agriculture varieties of winter wheat Claudius and Photinia are highly resistant to low temperatures.

Conclusions

Different frost resistance of varieties of winter wheat show a mixed biochemical response to low temperature stress, which reflects the degree of variability of the hydrolytic activity of the enzyme β-fructofuranosidase. I slaboprotochnyh varieties in critical temperature conditions, the activity of the enzyme increases to a maximum of 1.0 times, while hardy varieties, this figure rises to 4.2.

The resistance index that expresses the degree of activation of the enzyme β-fructofuranosidase the cooling of the sprouts, it is an objective measure of frost resistance of winter wheat varieties, as evidenced by the survival in the freezing cold room.

References

[1] Colosa OI. Cryopedological mechanisms of adaptation and resilience: Physiology and biochemistry cultivated plants, 1986, Vol. 18, No. 6; 555-567.

[2] Colosa OI, Kostenko II. the Water regime, enzymatic activity and frost-resistance of winter wheat: Reports of agricultural Sciences, 1973, No. 3; 11-14.

[3] Colosa OI. Method for determining the activity of the enzyme β-fructofuranosidase for comparative evaluation of frost resistance of winter wheat and rye. Kiev, 1976; 27.

[4] Kolupaev Yu, Sysoev LA, Manoylo GA, etc. On possible mechanisms of changes in the activity of invertase during cold hardening: Physiology and biochemistry cultivated plants, 1989, T. 21, No. 6; 560-566.

[5] Petrova OV, Kalosha OI, Mishustin SP, etc. Multiple forms of enzymes and modification of winter wheat in the period of adaptation to low temperatures: Physiology and biochemistry cultivated plants, 1985, 17, No. 4; 361-366.

[6] Presnyakov VE. Agroecological study of winter hardiness of varieties of winter wheat in forest-steppe zone of the Volga region: abstract of thesis of candidate of biological Sciences. Ramon, 2003; 24.

[7] Pochinok KhN. Methods biochemical analysis of plants. Kiev, 1976; 334.

[8] Savich IM. Peroxidase – stress proteins in plants: Advances of modern biology, 1989, T. 107, Vol.3; 406-417.

[9] Statsenko AP. Agro-ecological conditions for obtaining high yields of winter wheat in the foothills of the Northern Tien Shan: abstract of thesis of doctor of agricultural Sciences, Kiev, 1995; 41.

[10] Statsenko AP, Zolotukhin AI. Variability of the enzyme is a reliable indicator of the status of wintering of crops: Crops, 1994, No. 3; 12-13.

[11] Statsenko AP, Presnyakov EV. Biochemical control of frost resistance of winter crops: Advances in science and technology in agriculture, 2001, No. 1; 16-17.

[12] Chirkov AI. Grain crops in forest-steppe of the Volga region. Penza, 2003; 139.

ФЕРМЕНТАТИВНАЯ ДИАГНОСТИКА МОРОЗОСТОЙКОСТИ ОЗИМОЙ ПШЕНИЦЫ

Стаценко А.П., Блинохватов А.А.

Изучена изменчивость гидролитической активности фермента β-фруктофуранозидазы в проростках различных по морозостойкости сортов озимой пшеницы в процессе холодового закаливания и промораживания. Выявлена тесная сопряженность между температурным режимом среды, активностью фермента и морозостойкостью. Оценен уровень морозостойкости 15 сортов озимой пшеницы из различных экологических зон, в результате чего на основании индексов стойкости выделены 3 группы стойкости: морозостойкие - индекс стойкости 3,3-4,2 (Фотинья, Клавдия, Мироновская 808, Бирюза, Безенчукская 380); среднеморозостойкие – индекс стойкости 1,9-2,3 (Золушка, Смуглянка, Эритроспермум 2/01-3-04, Оренбургская 105, Кинельская 5); слабоморозостойкие – индекс стойкости 1,0-1,4 (Базальт, Лютесценс 2/01-17-04, Безенчукская 616, Эритроспермум 12/00-16-04, Санта). Гидролитическую активность фермента предлагается использовать в качестве оценочного показателя морозостойкости новых сортов озимой пшеницы. Степень изменчивости гидролитической активности фермента β-фруктофуранозидазы в проростках озимой пшеницы (индекс стойкости) позволяет вести объективную оценку на морозостойкость сортов и селекционного материала. Использование этого метода позволило установить, что созданные в Пензенском НИИСХ новые сорта озимой пшеницы Клавдия и Фотинья отличаются высокой стойкостью к низким температурам. Индекс стойкости, выражающий степень активизации фермента β-фруктофуранозидазы при охлаждении проростков, является объективным показателем морозостойкости сортов озимой пшеницы, что подтверждается выживаемостью при промораживании в холодильной камере.

Ключевые слова: *озимая пшеница, промораживание, морозостойкость, β-фрукто-фуранозидаза, индекс стойкости, группа стойкости, выживаемость, низкотемпературный стресс.*