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Biochemical adaptive plant response of corn lines with diferente drought tolerance

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ABSTRACT

The paper deals with impact of water deficit and heat shock on activity of sucrose-phosphate synthase (SPS) – (UDP-glucose: D-fructose-6-phosphat-2-α-glucosyltransferase, E.C. 2.4.1.14) – an enzyme involved in sucrose synthesis, on activity of soluble lectins, as well as sucrose and abscisic acid (ABA) content in germinating kernels of corn lines with different drought tolerance (Zea mays L.). The paper demonstrates that drought-tolerant corn lines are characterized by increased activity of soluble lectins, sucrose-phosphate synthase, abscisic acid and sucrose content in tissues of sprouts under the impact of adverse abiotic factors (water deficit and hyperthermia) compared to reference value.

Аннотация

Изучено влияние водного дефицита и теплового шока на активность сахарозофосфатсинтазы (СФС) - (УДФ-глюкоза D-фруктозо-6-фосфат-2-α-глюкозилтрансфераза, К.Ф. 2.4.1.14) - энзима синтеза сахарозы, активность лектинов, а также содержание сахарозы и АБК в прорастающих зерновках контрастных по признаку засухоустойчивости линий кукурузы (ZeamaysL.). Показано, что pfce [jecnjqxbdst линии кукурузы характеризуются повышением активности растворимых лектинов, сахарозофосфатсинтазы и содержания абсцизовой кислоты сахарозы в тканях проростков при воздействии абиотических неблагоприятных факторов (водного дефицита и гипертермии).

Keywords: *Zea mays L.*, soluble lectins, abscisic acid (ABA), sucrose-phosphate synthase (SPS), sucrose, water deficit, heat shock.

1. Introduction

One of the most pressing issues nowadays is a study of the plants' response to changes in meteorological conditions of environment. This problem is studied now by a new science – meteomics. Advantages of assessment of joint impact of meteorological factors on plants lie in the fact that such approach enables to simulate a real situation in nature.

Drought and high temperature are one of the key factors of the environment limiting crop capacity of grains. Response of the plants to drought and high temperature is very complex and includes interaction between various molecular, physiological and biochemical processes. Significant changes take place in hormone balance of plant cells under various stresses, contributing to change in structure and functions of plant cells under normal conditions into those under stress conditions. Abscisic acid (ABA) plays a key role in regulation of changes in gene expression of plant cells under stress. Under such conditions ABA level rises drastically, which leads to decreasing activity of metabolic processes in cells, namely, total protein synthesis, on the one hand, and induced creation of over a dozen of stress proteins, on the other hand [1, 2, 3].

Synthesis of a number of proteins present under normal conditions, including lectin, increases along with synthesis of stress proteins under adverse conditions, as well as treatment with exogeneous ABA [4, 5, 6]. This is supported by data concerning significant accumulation of lectin in roots of wheat sprouts under osmotic shock and drought, in sprouts in response to salinity of the environment, in cell culture under heat shock, as well as developing wheat kernels under water deficit [7, 8, 9]. Taking into account

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variety of lectins, their presence in various types of plants, and presence of various agglutinating proteins in organs of one and the same plant, one can assume that these molecules play an important physiological role in metabolism. One of the defence mechanisms of lectins under stresses of various nature is their possible impact on cytoskeleton's destabilization and stabilization cycle, which plays a key part in regulation of the plant's response to biotic and abiotic stimuli. Growth in lectin content may also lead to oxidative stress inhibition and decrease in active forms of oxygen, which along with stabilization of the cytoskeleton's new configuration takes the cell back to unexcited state. Based on the given data, lectin may be viewed as a party to nonspecific responses of the plants.

It is known that stress factors (drought, hypothermia, hyperthermia and others) may trigger both decrease and increase of soluble sugar content in cells. One of the most important osmoprotectors under stress conditions is sucrose. It is the main form of sugar transport in most plants. Moreover, sucrose serves as a sign for activation or repression of specific genes in various tissues [10, 11]. Since sucrose content in plants is determined by dynamic balance of its synthesis and hydrolysis, stress-induced change in sucrose content can be contributed to regulation of activity of enzymes synthesizing and engaging this disaccharide into metabolism. Sucrose-phosphate synthase (SPS – E.C. 2.4.1.13) is a key enzyme in sucrose biosynthesis. Major part of the SPS in sucrose accumulation is verified by genetic engineering methods [12]. SPS activity regulation under stress may be affected by metabolite accumulation level, genotypic features, plant's tolerance to an adverse factor [13, 14].

In consideration of the foregoing premises the goal of our study is to identify the patterns and characteristics of changes in the activity of soluble lectins, sucrose phosphate synthase, and the content of sucrose and abscisic acid in maize seedlings with different drought tolerance under water and heat stress in order to create new biochemical methods for assessing drought tolerance.

2. Material and Methods

Three-day young sprouts of corn lines (Zea mays L.) with different levels of drought tolerance were used in the research: drought-tolerant lines Od 329, IK107 zM, non-drought-tolerant lines GK 26, IK107VS3 / 66. Material was prepared and provided by A. O. Belousov, Dr. Sc. (Biology), Head of Laboratory of Genetic and Biotechnological Methods of Corn Selection of the Genetic Selection Institute – National Centre for Study of Seeds and Varieties. Undamaged corn kernels were used in the research. They were let germinate on filter paper in a germination chamber at 25o C at relative humidity 60%. Water deficit (WD) was created by placing the sprouts in the chamber with relative humidity 35-40%. Heat shock (HS) was created by placing the sprouts in the germination chamber at 37o C. Stress factors were applied for 6 hours. Plants in the reference group were subject to optimum humidity conditions at 25o C. Upon completion of exposure dissected aboveground parts of sprouts, endosperm and roots were frozen at -40o C. Lectin activity was determined on the basis of their ability to agglutinate trypsinized erythrocytes of white rats [15]. Total protein content in the extract was determined by Lowry protein assay [16]. Abscisic acid content was determined by gas chromatography [17]. Sucrose content was determined by gas chromatography [18]. Activity of sucrose-phosphate synthase (SPS) – (UDP-glucose: D-fructose-6-phosphat-2-α-glucosyltransferase, E.C. 2.4.1.14) was determined by the standard method [14]. Results were subject to mathematical and statistical processing in accordance with standard practices [18]. Data were expressed as means ± standard deviation (SD) for triple determinations.

3. Results

The following was found in the course of study of the

Table 1 | Induced Change of Activity Soluble Lectins in Sprouts of Corn Lines with Different Levels of Drought Tolerance. Note: * Probable difference P ≤ 0.05 against reference value. Индуцированное изменение активности растворимых лектинов в проростках линий кукурузы с различным уровнем засухоустойчивости. Примечание. * Отличия достоверны при p ≤ 0,05 относительно контроля.

Line	Lectin activity (μg protein/ml)$^{-1}$				% of reference value		
	Reference value	Water deficit	Heat shock	Water deficit + heat shock	Water deficit	Heat shock	Water deficit + heat shock
Drought-tolerant lines							
IK107 zM	14 ± 1	13 ± 1	38 ± 1*	34 ± 3*	97.4	276.0	244.1
Od329	16 ± 2	19 ± 1*	45 ± 2*	26 ± 1*	121.1	281.0	163.0
Non-drought-tolerant lines							
GK26 zM	6.0 ± 0.4	4.6 ± 0.2*	2.4 ± 0.1*	2.2 ± 0.2*	76.2	39.8	37.2
IK107VS$_3$/66	5.3 ± 0.1	3.5 ± 0.1	5.9 ± 0.3	2.5 ± 0.1	66.8	111.4	47.5

Table 1 | IAbscisic Acid Content in Corn Sprouts under Abiotic Stresses (mg/g). Note: * Probable difference P ≤ 0.05 against reference value. Содержание абсцизовой кислоты в проростках кукурузы при абиотических стрессах (мг / г.) Примечание. * Отличия достоверны при p ≤ 0,05 относительно контроля.

Lines	**Reference value**		**Water deficit**				**Heat shock**				**Water deficit + heat shock**			
	Above-ground part of sprout	Root	Aboveground part of sprout		Root		Aboveground part of sprout		Root		Aboveground part of sprout		Root	
	mg/g		mg/g	% of reference value	mg/g	% of reference value	mg/g	% of reference value	mg/g	% of reference value	mg/g	% of reference value	mg/g	% of reference value
Drought-tolerant lines														
IK107 zM	20 ± 2	9 ± 1	36 ± 3*	181.8	28 ± 2*	320.0	32 ± 3*	160.3	19 ± 1*	220	28 ± 1*	140.3	18 ± 1*	200.0
Od329	8 ± 1	10 ± 1	12 ± 1*	150.7	16 ± 1*	165.7	11 ± 1*	135.6	12 ± 1*	120.0	11 ± 1*	142.8	12 ± 1*	115.0
Non-drought-tolerant lines														
GK26 zM	17 ± 1	7.0 ± 0.4	9 ± 1*	58.8	4.0 ± 0.2*	54.3	14 ± 1	85.2	7.0 ± 0.4	98.2	12 ± 1*	75.0	6.0 ± 0.3*	88.8
IK107VS $_3$/66	5.0 ± 0.2	5.0 ± 0.3	4.7 ± 0.1	95.8	5.0 ± 0.2	99.6	5.0 ± 0.2	105.0	3.0 ± 0.1*	55.5	4.0 ± 0.2	95.0	4.0 ± 0.1	90.0

impact of stress factors, water deficit and high temperature on the total soluble lectin activity in tissues of whole sprouts and ABA content in tissues of the aboveground part and roots of 3-day young sprouts of corn lines with different levels of drought tolerance. The study enabled us to identify the increase in soluble lectin activity (160 – 280 % of reference value) and ABA content (135 – 320 % of reference value) under the given stress factors in drought-tolerant lines, and the decrease in soluble lectin activity (37 - 76 % of reference value) and ABA content (88 – 54,3 % of reference value, GK26zM) under the given stress factors in drought-tolerant lines (Tables 1, 2). Diverse changes in lectin activity under stress factors in plants with various levels of tolerance may occur due to synthesis of isoforms more adjusted to stresses, prevailing synthesis of which facilitates maintaining cellular metabolism at the necessary level. Moreover, reserve forms of lectin mRNA were found through inhibition assay when studying synthesis and accumulation of lectins in cells of the wheat germ [20]. The authors of the paper suggested that there was some kind of a pool of untranslated lectin mRNA and its precursors in wheat cells, and that a stress factor might contribute to mobilization of reserve forms of lectin mRNA into translation stage and trigger acceleration in processing of lectin precursors. It is probable that changes in lectin activity that we observed in tissues of the corn in response to the given stress factors are identified using the same or similar mechanism. One can assume that high level of induced accumulation of lectins in the aboveground part and roots of sprouts of drought-tolerant corn lines under stress factors may be connected with a higher speed of mobilization of reserve forms of lectin mRNA into translation stage, and, consequently, acceleration in processing of lectin precursors. Undoubtedly, this assumption requires verification by experiment and is a task of our further research. Transfer of plants into the state of nonspecific resistance, as well as many other processes connected with impact of stresses, frequently correlate with immunologically significant shifts in the plant's hormone system. In the first place it applies to abscisic acid (ABA), which is called a stress phytohormone. According to reference sources, increase in ABA content preceded increase in lectine content under stress factors of varying nature. Data on ABA and lectin concentration kinetics enabled us to conclude that there is a cause-effect relationship between stress-induced accumulation of ABA and increase in lectin content (activity). However, lectin activity and ABA content may be affected by other factors as well, first of all, genetic nature of plant lines and initial content of lectins and abscisic acid.

According to reference sources, sucrose-phosphate synthase activity and sucrose content may serve as auxiliary criteria for assessment of the plants' response to drought

Table 3 | Sucrose-Phosphate Synthase (SPS) Activity and Sucrose Content in Corn Sprouts Grown amid Water Deficit and Hyperthermia Note: * Probable difference P ≤ 0.05 against reference value. Активность сахарозофосфатсинтазы (СФС) и содержание сахарозы в проростках кукурузы, выращенных в условиях водного дефицита и гипертермии Примечание. * Примечание. * Отличия достоверны при p ≤ 0,05 относительно контроля.

Line	Sucrose-phosphate synthase activity(sucrose μmol / protein mg per hour)			Sucrose content (mg/g on dry basis)		
	Reference value	Water deficit + heat shock	% of reference value	Reference value	Water deficit + heat shock	% of reference value
Drought-tolerant lines						
Od 329						
Aboveground part	3.4 ± 0.1	4.8 ± 0.1*	143.5	15 ± 1	32 ± 3*	215.0
Roots	3.1 ± 0.1	6.7 ± 0.3*	217.2	11 ± 1	25 ± 1*	225.2
IK107 zM						
Aboveground part	3.8 ± 0.1	4.1 ± 0.1	110.0	19 ± 1	28 ± 2*	143.3
Roots	2.9 ± 0.1	3.3 ± 0.1	114.3	15 ± 1	21 ± 1*	139.2
Non-drought-tolerant lines						
GK26 zM						
Aboveground part	3.3 ± 0.1	2.56 ± 0.03*	76.6	14.0 ± 0.1	15.1 ± 0.1	107.4
Roots	3.1 ± 0.1	1.98 ± 0.03*	64.3	11.2 ± 0.1	13.3 ± 0.2*	119.3
IK107VS$_3$/66						
Aboveground part	5.7 ± 0.2	2.9 ± 0.1*	50.1	24.5 ± 0.4	26.8 ± 0.3	109.5
Roots	4.1 ± 0.2	1.98 ± 0.01*	48.1	23.7 ± 0.1	24 ± 1	100.6

tolerance only during a short drought, because a long drought inhibits the enzyme regardless of genotype tolerance to this stress. That is why sucrose-phosphate synthase activity and sucrose content were studied in sprouts of corn lines with different level of drought tolerance under a short joint impact of water deficit and hyperthermia. The study showed that sucrose-phosphate synthase was positively activated under stress factors in drought-tolerant corn lines both in the aboveground part and roots of the plants (143 - 217% of reference value – Od329, 110 - 114% of reference value – IK107zM) (Table 3). Stress factors in non-drought-tolerant corn lines inhibited sucrose-phosphate synthase activity in the aboveground part and roots of the plants (76,6 - 48% of reference value). Stress factors also affected the level of sucrose content in corn sprouts. For instance, activation of sucrose-phosphate synthase in drought-tolerant corn lines was accompanied by significant increase in sucrose content both in the aboveground part and in roots of the sprouts (140 - 225% of reference value). Inhibition of sucrose-phosphate synthase activity amid water deficit and hyperthermia in non-drought-tolerant corn lines had little effect on sucrose content in sprouts, though there was a tendency towards growth thereof (107 - 119% of reference value) (Table 3). In other words, activation of sucrose-phosphate synthase and sucrose accumulation amid water deficit and hyperthermia in corn depend on the genotype's drought tolerance and may serve as auxiliary criteria for assessment of prospective drought tolerance of corn lines.

4. Concluding Remarks

Therefore, it has been established that corn lines with positively different levels of drought tolerance are characterized by varying activity of soluble lectins, content of abscisic acid, activity of sucrose-phosphate synthase and sucrose content in tissues of the sprouts under adverse abiotic factors (water deficit and hyperthermia). Activation of soluble lectins, sucrose-phosphate synthase and accumulation of abscisic acid in corn amid water deficit and hyperthermia depend on the line's drought tolerance and may be used as biochemical criteria for assessment of drought tolerance of corn lines.

Заключение

Итак, установлено, что линии кукурузы, которые достоверно отличаются по уровню засухоустойчивости, характеризуются неодинаковыми активностью растворимых лектинов, абсцизовой кислоты, сахарозофосфатсинтазы и содержанием сахарозы в тканях проростков при воздействии абиотических неблагоприятных факторов (водного дефицита и гипертермии). Активация растворимых лектинов, СФС и аккумуляция абсцизовой кислоты в условиях водного дефицита и гипертермии в растениях кукурузы зависят от уровня засухоустойчивости линии и могут быть использованы в качестве биохимических критериев при оценке уровня засухоустойчивости линий кукурузы.

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