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COMPARATIVE STUDY OF BIOCHEMICAL PARAMETERS IN GENE-KNOCKOUT COTTON VARIETIES

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Abstract: It has been examined content of proline, fatty acids (FA) and carbohydrates in leaves of cotton "Cocker 312" (control) and gene-knockout varieties Porloq-1 and Porloq-2, obtained by RNA interference (RNAi) of phytochrome A1 (PHYA1) gene. The obtained results has shown that the composition FA and carbohydrates, and also proline content was significantly differed in Porloq-1 and Porloq-2 varieties compared to initial variety Coker-312.

Key words: cotton, PHYA1, RNAi, proline, fatty acids, carbohydrates.

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At the present time, soil drought and salinity are the mayor factors decreasing crop yield (Kumar et al., 2018). This phenomenon is dramatized by both climate changes and intensification of agronomy technologies. However, plants have different defense mechanisms on various levels: cellular, organs and organism as whole (Kumar et al., 2018; Hamed et al., 2018; Nouman et al., 2018; Saini et al., 2018). Herewith it is known that abiotic stresses change a number of biochemical parameters of cotton leaves, such as FA composition (Zhang et al., 2013), proline content (Zhang et al., 2014), water-soluble sugars and starch (Peng et al., 2016), and these parameters can also be used for the selection of stress-resistant varieties in the cotton breeding.

Taking into account all the above, as well as basing on data about the resistance of gene-knockout cotton varieties of the Porloq series to abiotic stresses (Abdurakhmonov et al., 2014), we have carried out comparative study of the biochemical composition of the leaves of gene-knockout and unmodified cotton varieties.

Materials and methods.

The study object and experiment design.

Plants were grown under standard conditions in a phytotron. Pre-treated and sterilized cotton seeds, Gossypium hirsutum L., were planted in a sterile nutrient medium. Seeds were incubated in dark conditions and at a temperature of 28° C for three days. Three days later, Petri dishes with germinated seeds were converted into photoperiodic light/dark conditions with a duration of 16/8 hours, respectively. The power of light was equal to 5000 lux. After the appearance of 3-4 true leaves of the plant was transferred to the soil. All plants were subjected to genetic verification to confirm the presence of the RNAi construct.

Determination of carbohydrate content. Carefully weighed leaves (1,0 g) was placed in a 10 ml centrifuge tube and mixed with 5 ml of 80% ethanol. The mixture was incubated in a water bath at 80° C for 30 minutes with constant shaking, centrifuged at 4000 g for 5 minutes and the supernatant was collected. The granules were exposed to two extractions using 80% ethanol. All supernatants were combined and diluted to 25 ml with 80% ethanol, mixed and stored at -20° C to measure soluble sugar and sucrose.

The insoluble in ethanol residue was subjected to starch extraction. After evaporation of the ethanol the starch was dissolved in 2 ml of distilled water by boiling for 15 minutes and cooled to room temperature. Then the starch was hydrolyzed with 9.2 M HClO4 (2 ml) for 15 minutes, diluted with 4 ml of distilled water and centrifuged at 4000 g for 10 minutes. The precipitate was extracted once using a 4.6 M HClO4 solution (2 ml). Supernatants were left to stand, combined and diluted with distilled water to 25 ml.

The content of soluble sugars and starch in the collected extracts was determined by the anthrone method (Seifter et al., 1950). The sucrose content was analyzed in the resuspended supernatant according to the previously described protocols (Hendrix, 1993).

FA determination. Lipids were methyl-esterified with a 0.4 M KOH containing methanol and a mixture of equal volumes of benzene and petroleum ether (1 : 1, v/v), according to the method of Yu and Su (1996). The FA methyl esters were separated by gas chromatography on a gas chromatograph (Shimadzu GC-17A or similar), equipped with a hydrogen flame detector and a SP-2330 capillary column (15 m ? 0.32 mm). The temperature of the column is isothermal is 1650 C, the temperature of the detector is 2500 C. The double bond index (DBI) was used to measuring of the unsaturation degree of FAs.

Free proline determination. Proline content was determined by reaction with ninhydrin (Bates et al., 1973). For colorimetric determinations, a solution of proline, ninhydric acid, and glacial acetic acid (1: 1: 1) was incubated at 90° C for 1 h. Then the reaction mixture was cooled in ice bath. The chromophore was extracted using 2 ml of toluene, and its absorbance was determined on a suitable spectrophotometer at 520 nm.

Statistical analysis. All data were subjected to variance analysis using the OriginPro 7.0 software. Data are presented as mean ë standard error. The significance of the differences between the mean values was determined using the Tukey test. Differences at p ? 0.05 and p ? 0.01 were considered as significant.

Results and discussion. At the first stage, we have studied the content and composition of phospholipids, which are responsible for membrane stability both under normal conditions and under stress (Hou et al., 2016; Sui et al., 2018). As it can be seen from the table. 1, FAs composition was different in various cotton genotypes.

The main FA in all studied samples were palmitic acid (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3). These five FAs have figured up to more than 95% of the total FA content in functional cotton leaves (Table 1). At the same time, the FA composition in RNAi varieties has a higher percent of unsaturated FAs (UFA) and a lower - saturated FAs (SFA) as compared to the control unmodified Coker-312 genotype.

In the early stages of stress, the production of ROS as signaling molecules initiates plant defense reactions (Noctor et al., 2014). ROS are produced in distortion of electron transfer in photosystems I and II (Noctor et al., 2014; Caverzan et al., 2016). These oxyradicals initiate lipid peroxidation in which FA radicals are formed (L-, LO- and LOO-). FA radicals are not stable, so they easily react with molecular oxygen, thus turning into various lipid peroxides and, ultimately, are converted into

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MDA and other aldehydes, short-chain ketones, carboxylic acids and hydrocarbons (Caverzan et al., 2016). These series of reactions cause a decrease in the content of FA and an increase in the content of MDA.

Many plants accumulate proline as a non-toxic and protective osmolyte, both under physiological conditions and under osmotic stress which associated with lack of moisture and/or salinity (Hayat et al., 2012). In this regard, the effect of silencing of PHYA1 gene on the content of free proline in the leaves of various cotton varieties was studied. As can be seen from Fig. 1, the content of proline in different cotton varieties was significantly different and correlated with presence of vector construct in genome and also their resistance to water deficiency and salinity. In this case, the smallest amount of proline was observed in the unmodified initial variety Coker-312, and the highest in RNAi variety Porloq-2.

This accumulation of osmolytes, especially proline, is usual in plants. In addition to its role as an osmolyte, proline promotes removing of ROS, stabilizing subcellular structures, modulating the redox homeostasis of cells, providing energy and functioning as a signal (Gharsallah et al., 2016). Although the accumulation of proline is a common reaction to salt stress in plants, the degree of its accumulation varies between tolerant and sensitive genotypes. Indeed, our results showed that proline accumulation was significantly increased in the leaves of resistant genotypes compared to sensitive genotypes.

Drought and salt stress is known to inhibit plant growth, mainly by suppressing leaf growth and reducing photosynthesis rate (Pn) (Peng et al., 2016). The decreasing of Pn is essentially associated with the stomatal function or increasing of sucrose content in the leaves (Peng et al., 2016). For example, it has been shown that the differences between salt-sensitive and salt-resistant varieties of chickpeas are related to reducing of photosynthesis due to damaging of photosystem II (Khan et al., 2015). Moreover, the content of primary metabolites of amino acid and carbohydrate metabolism are increased in resistant plants, and these solutes are responsible for osmotic regulation, protection of membranes and proteins, or in ROS uptake (Peng et al., 2016).

Like the most plants, sucrose and starch are the main photosynthesis products in cotton. Moreover, the sucrose content is known to be sensitive to abiotic stresses (Peng et al., 2016). Taking into account all the above, as well as the fact that the Porloq cotton varieties are more resistant to abiotic stresses (Abdurakhmonov et al., 2014), we have studied the carbohydrate content of various cotton genotypes.

As can be seen from Fig. 2, the differences in content of soluble sugars, sucrose, starch and the ratio of sucrose / starch in the leaves have depended on the presence of a vector

construct in cotton genome. Herewith, the RNAi cotton varieties (Porloq-1 and Porloq-2) have a higher content of soluble sugars and sucrose and, accordingly, a higher value of the ratio of sucrose / starch compared to the non-transformed cotton variety Coker-312 (Fig. 2). These results have well correlated to the available literature data on the sugar content of various cotton genotypes under salt stress conditions (Peng et al., 2016).

Like other higher plants sugars (in particular, starch and sucrose) are the main photosynthesis products in cotton (Huber and Huber, 1996). At the same time, soluble sugars are very sensitive to abiotic stresses (Peng et al., 2016). However, it should be noted that sugars provided cell by energy and soluble substances for osmotic regulation, and also change the expression of many genes as secondary messengers (Peng et al., 2016).

In this regard, the obtained results allow us to suggest that the distribution of photosynthetic products in RNAi varieties is favorable to the sucrose synthesis in the stem leaves, which may be responsible for a change in the activity of enzymes that provide sucrose metabolism.

Thus, summarizing the above, it should be noted that the phenotypic resistance of RNAi cotton varieties Porloq to abiotic stresses is confirmed by molecular studies. A higher content of free proline, soluble sugars and sucrose was observed in these varieties and, accordingly, a higher sucrose / starch ratio compared to the unmodified cotton variety Cocker-312.

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References:

1. Abdurakhmonov, I.Y., Buriev, Z.T., et al. (2014). Phytochrome RNAi Enhances Major Fibre Quality and Agronomic Traits of the Cotton Gossypium hirsutum L. Nature Communications, 5: 3062.

2. Bates, L.S., Waldren, R.P., Teare, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.

3. Caverzan, A., Casassola, A., Brammer, S.P. (2016). Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. In: Shanker A, Shanker C (eds) Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives, Croatia. InTechOpen, pp. 463-480.

4. Gharsallah, C., Fakhfakh, H., et al. (2016). Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AoB Plants, 8: 1-21.

5. Hamed, K.B., Dabbous, A., et al. (2018). Salinity Responses and Adaptive Mechanisms in Halophytes and Their Exploitation for Producing Salinity Tolerant Crops. In: Kumar V. et al. (eds) Salinity Responses and Tolerance in Plants: Exploring RNAi, Genome Editing and Systems Biology, Switzerland: Springer Nature, pp. 1-20.

6. Hayat, Sh., Hayat, Q., et al. (2012). Role of proline under changing environment: A review. Plant Signaling & Behavior, 7 (11): 1-11.

7. Hendrix, D.L. (1993). Rapid extraction and analysis of nonstructural carbohydrates in plant tissues. Crop Sci, 33(6): 1306-1311.

8. Hou, Q., Ufer, G., Bartels, D.(2016). Lipid signalling in plant responses to abiotic stress. Plant, Cell and Environment, 39: 1029-1048.

9. Huber, S.C. and Huber, J.L. (1996). Role and regulation sucrose phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol., 47 (1): 431-444.

10. Khan, H.A., Siddique, K.H., et al. (2015). Salt sensitivity in chickpea: Growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes. J Plant Physiol 182: 1-12.

11. Kumar, S., Sachdeva, S., et al. (2018). Plant Responses to Drought Stress: Physiological, Biochemical and Molecular Basis. In: Vats Sh. (ed) Biotic and Abiotic Stress Tolerance in Plants, Singapore: Springer Nature Singapore Pte Ltd, pp. 1-26.

12. Noctor, G., Mhamdi, A., Foyer, C.H. (2014). The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol., 164: 1636-1648.

13. Nouman, W., Qureshi, M. K., et al. (2018). Variation in Plant Bioactive Compounds and Antioxidant Activities Under Salt Stress. In: Vats Sh. (ed) Biotic and Abiotic Stress Tolerance in Plants, Singapore: Springer Nature Singapore Pte Ltd, pp. 77-102.

14. Peng, J., Liu J., et al. (2016). Effects of Soil Salinity on Sucrose Metabolism in Cotton Leaves. PLoS ONE, 11(5): e0156241.

15. Saini, P., Gani, M., et al. (2018). Reactive Oxygen Species (ROS): A Way to Stress Survival in Plants. In: Zargar SM, Zargar MY (eds) Abiotic Stress-Mediated Sensing and Signaling in Plants: An Omics Perspective, Singapore: Springer Nature Singapore Pte Ltd, pp. 127-154.

16. Seifter, S., Dayton, S., et al. 1950. The estimation of glycogen with the anthrone reagent. Arch. Biochem 25(1): 191-200.

17. Sui, N., Wang, Y., et al. (2018). Transcriptomic and Physiological Evidence for the Relationship between Unsaturated Fatty Acid and Salt Stress in Peanut. Front. Plant Sci., 9: 7.

18. Yu, H.G. and Su, W.A. (1996). Studies on relationship between fatty acids desaturation of PSII membrane and low temperature photoinhibation of cucumber. Acta Biochimica Biophysica Sinica, 12: 227-233.

19. Zhang, L., Ma, H., et al. (2014). Morphological and Physiological Responses of Cotton (Gossypium hirsutum L.) Plants to Salinity. PLoS ONE, 9(11): e112807.

20. Zhang, L., Zhang, G., et al. (2013). Effect of soil salinity on physiological characteristics of functional leaves of cotton plants. J Plant Res., 126: 293-304.

Table 1. Fatty acid composition in various genotypes of cotton.

	Coker-312	AN-Bayavut-2	Porloq-1	C-6524	Porloq-2
C16:0	20.47±0,9	21.55±1,0	22.90±1,12*	21.93±1,04	22.77±0,82*
C18:0	2.39±0,1	3.11±0,15	3.59±0,21**	1.22±0,21	2.36±0,09*
C18:1	4.88±0,2	3.36±0,23	7.01±0,37*	4.41±0,15	5.02±0,23*
C18:2	11.24±0,5	8.99±0,41	8.41±0,35*	9.33±0,51	9.58±0,44*
C18:3	61.02±1,2	62.99±1,24	58.09±1,18*	63.11±1,23	60.27±0,84*
SFA	22.86±0,8	24.66±0,83	26.49±0,56**	23.15±0,94	25.13±0,45**
UFA	77.14±1,6	75.34±1,56	73.51±1,32**	76.85±1,64	74.87±1,58*
UFA/SFA	3.37±0,2	3.05±0,14	2.78±0,15*	3.30±0,21	2.97±0,18**

SFA – saturated FAs (C16:0 + C18:0), UFA – unsaturated FAs (C18:1 + C18:2 + C18:3), *UFA/SFA* = (C18:1 + C18:2 + C18:3)/(C16:0 + C18:0)
* significance of differences (*P < 0.05; **P < 0.01)

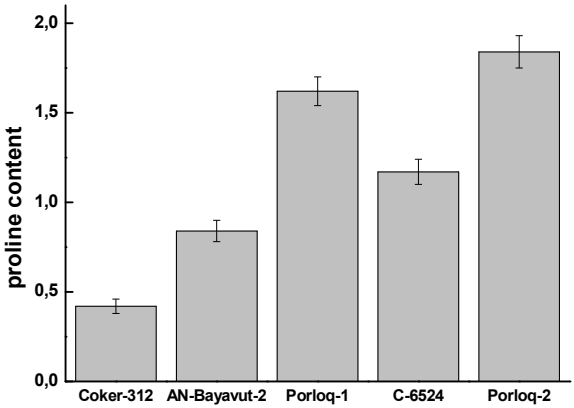

Fig. 1. The content of free proline in the leaves of various genotypes of cotton. On the X-axis – the investigated varieties; the Y-axis – proline content, in µg/g wet weight. $p \leq 0,05$.

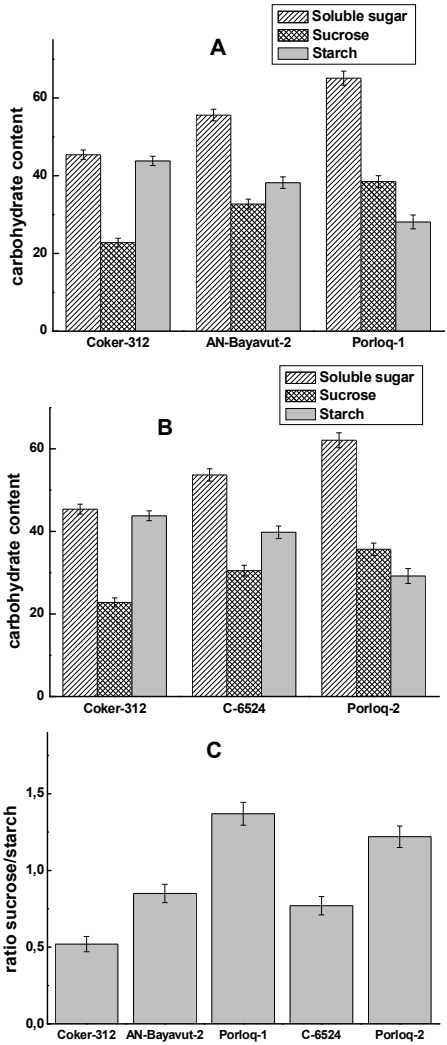

Fig. 2. Carbohydrate content in stem leaves of various cotton genotypes. On the X-axis – the investigated varieties; the Y-axis – carbohydrate content, in mg/g of wet weight (A, B) and sucrose / starch ratio (C). $p \leq 0,05$.