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INFLUENCE OF EXOGENOUS ABSCISIC ACID ON THE LEVELS
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ABSTRACT

In this article, the exogenous effect of abscisic acid on the concentration of reducing sugars and proline in seedlings of Porloq-1 and Ravnaq-1 cotton varieties created by methods such as gene knockout and MAS technology was studied over time (after 1 and 24 hours). As a result of the exogenous action of abscisic acid, the concentration of reducing sugars and proline increased with increasing salinity. Exogenous abscisic acid pre-treatment can reduce the damaging effects of salt stress by enhancing the biosynthesis of photosynthetic pigments and thereby increasing the general growth rate.

АННОТАЦИЯ

В данной статье изучено экзогенное влияние абсцизовой кислоты на концентрацию редуцирующих сахаров и пролина в проростках сортов хлопчатника Порлок-1 и Равнак-1, созданных такими методами, как нокаут генов и технология МАС в динамике (через 1 и 24 часа). В результате экзогенного действия абсцизовой кислоты концентрация редуцирующих сахаров и пролина увеличивалась с увеличением солености. Предварительная обработка экзогенной абсцизовой кислотой может уменьшить повреждающее действие солевого стресса за счет усиления биосинтеза фотосинтетических пигментов и тем самым увеличения общей скорости роста.

Keywords: salinity; abiotic stress; phytohormone; abscisic acid; osmolyte; reducing sugars; proline; MAS technology; gene-knockout technology.

Ключевые слова: засоления, абиотический стресс, фитогормон, абсцизовая кислота, осмолит, редуцирующие сахара, пролин, МАС технология, ген-нокаут технология.

Soil salinity is a major environmental limitation to global agriculture, affecting 800 million hectares of land. This estimates over 6% of the world's total land area, affected either by salinity (397 million hectares) or

associated forms of sodicity (434 million hectares) [1]. Salt stress alters various biochemical and physiological responses in plants and thus affects almost all plant pro-

cesses, including photosynthesis, growth, and development [2]. Studies on salinity stress will be a stride towards the urgent need to develop crop varieties possessing higher growth rates and yields in salt-affected environments.

The involvement of phytohormones in the resistance of cotton plants to salt stress is one of the significant points that require deeper knowledge. Phytohormones play an important role in various biochemical and physiological mechanisms in plants. Their role in alleviating abiotic stress (such as salinity) is critical in providing tolerance to plants under adverse conditions. To improve the adverse outcome of salinity stress on plant growth, diverse phytohormones are extensively used, and one such phytohormone is abscisic acid (ABA), a plant stress hormone that is considered an important agent in the mechanisms of resistance and adaptation in plants against salt stress conditions [3]. ABA was also found to be involved in several other physiological processes, such as stomatal closure, embryo morphogenesis, development of seeds and synthesis of storage proteins and lipids [4], germination leaf senescence, and defence against pathogens [5]. Plants accumulate compatible osmolytes such as proline and reducing sugars when they are subjected to salinity stress, and they appear to protect plants from such stresses [6]. Osmolytes are known to function in protecting macromolecules by stabilizing protein structure and/or scavenging ROS produced under stress conditions [7]. Proline is a dominant organic molecule that acts as a mediator of osmotic adjustment under salinity stress, a stabilizer of subcellular structures, a sink for energy, and even a stress-related signal. It is also involved in cell osmoregulation and the protection of proteins during dehydration and can act as an enzymatic regulator during stress conditions [8].

Plants accumulate osmolytes or compatible solutes to protect the cellular machinery from various environmental stresses. Reducing sugars are one of the most important osmolytes and protect plant cells from oxidative damage. Abscisic acid promotes the biosynthesis of reducing sugars in plants under abiotic stress conditions and helps improve plant tolerance against abnormal growth conditions. Reducing sugar-related compounds that act as osmoprotectants such as thiols and trehalose also accumulate in plant cells to regulate cellular osmotic adjustments under stress conditions. ABA signalling is also involved in the regulation of sugar metabolism in plants. Glucose and fructose levels are enhanced under water deficit conditions, accompanied by enhanced vacuolar invertase activity, and gene expression of invertase enzyme (IVR2) is enhanced by ABA [9]. Farooq and

Bano [10] mentioned that ABA regulates soluble sugar accumulation by modulating the activity of the amylase enzyme under stress conditions. Since both osmolytes and plant hormones have been known to play major roles during challenging environments, it is therefore imperative to correlate both and further elucidate the role of phytohormones in the regulation of osmolytes under abiotic stress.

Materials and methods

The objects of research were biotechnological varieties of cotton bred by the methods "gene knock" - Porlok-1 and MAS (a marker of associated selection) Ravnak-1. The Center of Genomics and Bioinformatics of the Academy of Sciences of the Republic of Uzbekistan provided the seeds. The seedlings were grown with tap water, and then the samples were subjected to salt stress by exposing them to a solution of 1% and 4% NaCl for 1 hour and then for 24 hours. A 10^{-7} M concentration of exogenous ABA was used in the experiment. Thus, the seedlings were used for further analyses. Control seedlings were germinated in water.

Determination of the content of reducing sugars was carried out according to the Shomody-Nelson method [11].

The method of Bates [12] was used for the determination of proline.

The results were processed using Excel. The mean deviation index ($\pm M$) and statistical reliability index (P) were determined, and results less than $P < 0.05$ were considered statistically significant.

Results and discussion

Phytohormone ABA promoted the accumulation of reducing sugars in cotton varieties Porlok-1 and Ravnak-1 under prolonged exposure to salt stress (Table 1 and 2). Thus, the Porlok-1 variety showed an increase in the content of reducing sugars in the first hour of exposure to 4% NaCl by 3.6 times compared to the control. At 24 hours of exposure to the variants with 1% NaCl and 1% NaCl + ABA, the content of reducing sugars increased 3.9- and 3.4-fold, respectively, compared with the control. The study of the content of reducing sugars in the Ravnak-1 variety at the first hour after exposure to 1% NaCl and 1% NaCl + ABA showed an increase in the content of sugars, the values of which exceeded the control by 1.9 and 1.3 times, respectively. In cultivar Ravnak-1, a high content of reducing sugars was also observed after 24 hours of exposure under the action of 4% NaCl + ABA; an increase of 2.3 times was found in comparison with the control.

Table 1.

The concentrations of reducing sugars in cotton seedlings under chloride salinity (mg/gr)

Treatment	Porlok-1		Ravnak-1	
	after 1 hour	after 24 hours	after 1 hour	after 24 hours
Control	0.85±0.03	0.85±0.03	1.86±0.08	1.50±0.03
1% NaCl	0.76±0.02	1.26±0.05	1.66±0.04	1.60±0.01
1% NaCl + ABA	0.96±0.04	1.40±0.06	1.14±0.04	1.20±0.01
4% NaCl	0.78±0.03	0.53±0.02	1.06±0.03	0.93±0.03
4% NaCl + ABA	0.81±0.02	0.73±0.02	1.13±0.02	2.24±0.08

The present study revealed that the Ravnak-1 variety of cotton accumulated more proline than the Porlok-1 variety (Table 2). Especially after 1 hour of exposure to stress, Ravnak-1 accumulated 2.3 and 1.7 times more proline, respectively, than Porlok-1 under conditions of 4% NaCl and 4% NaCl + ABA. After 24 hours of exposure

to stress, a sharp decrease in proline was observed in the Porlok-1 variety of cotton. The accumulation of proline in the Ravnak-1 variety also increased with increasing salinity. In particular, Ravnak-1 was found to contain five times more proline in a 4% NaCl medium than Porlok-1.

Table 2.

The concentration of proline in cotton seedlings under chloride salinity (mkg/gr dry matter)

Treatment	Porlok-1		Ravnak-1	
	after 1 hour	after 24 hours	after 1 hour	after 24 hours
Control	33.55±1.5	33.55±1.5	207±8.5	208±8.0
1% NaCl	81.4±3.5	96.06±4.3	211±4.2	314±12.8
1% NaCl + ABA	34.4±1.1	82.5±2.8	187±7.4	255±10.5
4% NaCl	33.6±1.0	27.8±1.3	252±10.6	380±14.0
4% NaCl + ABA	52.2±2.5	62.7±2.6	280±12.4	84±3.2

The accumulation of proline under salinity stress has been reported in *Pisum sativum* [13], *Brassica juncea* [14], and *Triticum aestivum* [15]. Proline accumulation is possible due to an increase in the enzymes involved in its synthesis or a decrease in the enzymes involved in its oxidation. Parida et al. [16] stated that there is a strong association between increased cellular proline levels and the capacity to survive high environmental salinity. The seedlings treated with ABA + NaCl exhibited higher adaptive potential under salinity stress, as expected by photosynthetic performance, antioxidant defence metabolism, and accumulation of osmoprotectants, than the control seedlings. Proline may also be the major source of energy and nitrogen during immediate post-stress metabolism. As a well-known fact, accumulated proline supplies energy for growth and survival, thereby inducing salt tolerance. The results also highlight that the seedlings accumulated more proline in addition to salinity stress. Similar findings were reported by Hassine and Lutts [17]. Stewart [18] reported that ABA stimulated proline synthesis from glutamic acid. In contrast, McDonnell et al. [19] reported that exogenous ABA treatments did not affect proline accumulation in *Spinacia* or *Pennisetum* seedlings.

In the cotton varieties that we studied, there was a tendency for a high accumulation of reducing sugars and proline under prolonged exposure to salts and the complex action of ABA phytohormones with salinity.

Conclusion

Thus, the Ravnak-1 cotton variety created from MAS technology is sensitive to salinity in combination with phytohormones, and the concentration of reducing sugars and proline increases depending on the duration of salt stress. In the Porlok-1 variety, under the influence of salinity, the concentrations of reducing sugars and proline increased insignificantly due to the inherent adaptive capacity of the variety. The cotton variety Porlok-1, created based on gene knockout technology, is a salt-tolerant variety.

Exogenous abscisic acid pre-treatment can reduce the damaging effects of salt stress by enhancing the biosynthesis of photosynthetic pigments and thereby increasing the general growth rate. Therefore, the exogenous application of ABA in crop plants may play a vital role and can act as an alternative way to improve crop productivity when cultivated under salt stress.

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