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THE INFLUENCE OF SALICYLIC ACID ON THE METABOLISM OF CARBOHYDRATES ON WHEAT AND CORN UNDER DROUGHT CONDITIONS

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The effect of salicylic acid and drought on wheat and corn is the subject of this study. The effects of drought stress with salicylic acid (SA) on some physiological and biochemical parameters of maize plants (Zea mays L.) of Zovta Zubovidna variety and wheat (Triticum aestivum L.) of Podolyanka variety were investigated. There was observed the increase in the amount of sugar in the corn leaves both under short-term and long-term drought conditions. Given the full recovery of soil moisture capacity (55–60 %) the amount of fructose in the corn plant tissues turned out to be higher than in the control sample but much lower than in the plants affected by drought. Prestress treatment of the corn seeds with salicylic acid caused the increase in the total amount of sugar. Salicylic acid caused increase of the total carbohydrates content in wheat, which were grown under optimal water supply (60 % of full moisture capacity). At the same time in the shoots of wheat, which seeds were not treated with salicylic acid, we observed, that accumulation of fructose was significantly higher compared with control. The results revealed that salicylic acid in the concentration 50 mM can considerably alleviate the damage triggered by the drought stress.

Keywords: drought, salicylic acid, carbohydrates, fructose, *Triticum aestivum* L., *Zea mays* L.

Drought is a major environmental problem, which hampers a number of physiological and metabolic processes in plants and may lead to suppressed its growth and development, reduced crop yield, or even can cause plant death. Indifferent plant species drought imposes various physiological and biochemical limitations and adverse effects [19]. To improve crop productivity, it is necessary to understand the mechanism of plant responses to drought conditions with the ultimate goal of improving crop performance in different parts of our country where rainfall is limited or unreliable. In addition to the complexity of drought itself, plant's responses to drought are complex and different mechanisms are adopted by plants when they encounter drought. One of the mechanisms utilized by the plants to overcome water deficiency stress effects might be the accumulation of compatible osmolytes, such as soluble sugars, free amino acids, proline and etc [22]. It is reported that drought stress causes changes in the amount of carbohydrates in the plants and with increasing drought stress, on leaves the amount of starch decreases [27].

The commercial form of salicylic acid (SA) is acetylsalicylic acid. It is known that in aqueous solutions, acetylsalicylic acid is hydrolyzed almost entirely to salicylic acid, which is its active ingredient. Depending on its concentration, salicylic acid performs important actions in the growth and development of plants [29]. These actions include a thermogenic effect, increasing thermotolerance. This acid has a herbicidic effect, providing resistance against pathogens and enhance the tolerant ability of the plant to drought stress [20].

SA plays an important role in abiotic stress tolerance, and more interests have been focused on SA due to its ability to induce a protective effect on plants under adverse environmental conditions. SA may affect directly enzymes function or may activate the genes that are responsible for protective mechanisms [22]. Shruti and Singh [24] showed that salt-induced deleterious effects in maize seedlings were significantly eliminated by the pretreatment of SA. It is concluded that 0.5 mM SA improves the adaptability of maize plants to NaCl stress [29].

Gunes [6] reported that SA could be used as a potential growth regulator that improve plant salinity tolerance. Several methods of application (soaking seeds in SA prior to sowing, adding SA to the hydroponic solution, irrigating or spraying with SA solution) have been shown to protect various plant species against abiotic stress, such as salinity [26].

SA is a plant growth regulator (PGR) that is part of a signaling pathway induced by several biotic and abiotic stresses. It has been identified as an endogenous regulatory signal that mediate plants defense against pathogens [10], and it is also a natural signal molecule that take part in activation of plant's general defense mechanisms. Exogenous application of SA has been shown to induce plant stress tolerance. A number of research demonstrates that exogenous SA have protective effects on against salinity [25], drought [9], and high temperatures [2].

Water deficiency has an impact on the physiological and biochemical processes, including carbohydrate metabolism which plays a prominent role in the adaptation of the plants to stress. The accumulation of soluble carbohydrates, the activity of enzymes that are involved in their synthesis and metabolism are determined by the type of the plant, its particular to adapt to stress ability. Water deficit stress is marked by the activation of neutral invertase, which hydrolyzes disaccharides [21]. It is believed that drought may be associated with physiological parameters, such as relative water content, relative water loss and drought susceptibility index of isolated leaves [12].

Water stress affects many physiological and biochemical processes in plants [4, 5, 17], resulting in the alteration of some metabolic pathways. Among the major effects are those involving carbohydrate metabolism, with the accumulation of sugars and a number of other organic solutes [18]. Sugars have long been known to increase in a wide range of plants grown at low moisture levels and under salinity, e.g. in wheat [10], pasture grasses [23] and cotton [25]. The rate and extent of increase in sugar content depends on the environmental conditions, species, and even on the genotype within the same species. Pirasteh [19] pointed out that the majority of xerophytes have the ability to produce a high content of sugars in dry habitats, whereas mesophytes accumulate them far less.

Although the increase in sugar content in response to water stress is well known, few reports indicate the changes in sugars concentrations at different levels of stress and recovery. Three principal sugars such as glucose, fructose and sucrose accumulate in crop plants but little information relative to which of these is most involved in water stress is available [3, 17]. The aim of the present work was to study the relationship between sugar content and water status during the development of water stress and after rewatering of wheat and corn.

Materials and methods

The objects of our study were plants of corn (*Zea mays* L.) of Govta Zubovidna variety and wheat (*Triticum aestivum* L.) of Podolyanka variety. Pre-seed soaked in a solution of SA (50 mM) for 3 h. First seeds germinated in an incubator, and on the 3rd day of growth were transplanted into plastic pots (d = 14 cm). Plants were grown on soil substrate, whose humidity was maintained at 60 % of full moisture capacity – optimal water supply. The model of drought was created by the simultaneous cessation of irrigation (30 % of soil moisture capacity) during 12 days. Upon termination of drought, soil moisture in the pots was adjusted to 60 % of full capacity. The control plants were grown from the seeds not treated with salicylic acid under conditions of optimal water supply (60 %). For our investigation samples were taken from the shoots of wheat and corn on 7, 9 and 12-days of drought period and on the first day 1 h after the resumption of irrigation (14-days).

Phenol-sulphuric method [3]. Sensitivity of this method ranges from 10 to 100 µg of total sugar amount. The evaluation was made on the basis of the calibration curve using glucose as a standard. The calculations were performed by means of the equation of linear regression obtained from the calibration curve. Given an aliquot of 100 µg mL^{-1} from the sample, we added 0.5 mL of 5 % phenol solution, shook it in vortex, added 2.5 mL, shook it again and kept in the water-bath at temperature of 25 °C for 15 minutes. After that, the absorbance were measured by the spectrophotometer at 490nm wave length.

Sulphuric Acid-Cysteine-Tryptophan method [16]. The sensitivity of the method ranges from 1 to 50µg of fructose. A standard solution of fructose was used to build the calibration curve and to get the equation of the linear regression to quantity samples. Given an aliquot 50 µg mL^{-1} of the sample, we added 2.8 mL of 75 % sulphuric acid, shook it in vortex, added 0.1 mL 2.5 % cysteine hydrochloride solution, shook it in vortex again, put it in the water bath at temperature of 45–50 °C for 10 minutes, cooled to room temperature and added 1 mL of tryptophan hydrochloride solution, then shook it again in vortex. After that, we read the absorbance on the spectrophotometer of 518nm wave length.

Results and discussions

Drought is an important factor that could influence the growth and physiological characteristics of plants [18, 28]. The responses of plants to drought stress depend on the species and genotype, the length and severity of water deficiency and the plant age and its stage of development [15, 21]. In this study, we investigated the effect of salicylic acid to the severity of drought stress on the wheat and corn.

The accumulation of sugars in response to drought stress is well documented [17]. A complex essential role of soluble sugars in plant metabolism is well knownnot only as products of hydrolytic processes and energy production but also in a sugar sensing and signaling systems. Soluble sugar may also function as a typical osmoprotectant, stabilizing cellular membranes and maintaining turgor pressure [7, 8].

In course of the research there has been observed the increase in the amount of carbohydrates in the leaves of wheat both under short-term and long-term drought conditions (Fig. 1). The content of the carbohydrates in shoots of the plants increases significantly in the seeds not treated with SA. These results are consistent with the studies which reported that drought stress causes increase in sugar content [9, 12]. The aleviation of sugar can be a result of photosynthesis reduction, because reduction of water causes raises of torger and losing torger pressure causes clothing stoma and finally decreasing photosynthesis. Thus, pretreatment with SA has caused a significant increase in sugar content in wheat plants on 7, 9, 12 and 14th days of growth under drought conditions compared to plants, which seeds not treated with SA. Treatment with salicylic acid enhances resistense of the plants to stress and as a result sugar approaches its norm.

The corn plants showed a slightly different effect of salicylic acid under drought conditions (Fig. 2). The concentration of total soluble sugars in shoots was significantly affected by drought. For example, on the 7^{th}, 9^{th} and 12^{th} day of water deficiency we observed increase in sugar content. However, under short- and long-term drought conditions there was observed the increase in the total amount of soluble sugars in the tissues of corn that were pretreated with a solution of salicylic acid compared to plants of corn, which seeds not treated with SA. Thus, the protective effect of SA on the impact of drought is closely linked to changes in metabolism of plants.

Fructose content showed a very similar pattern, also well correlated with drought stress. The correlations observed in the present study, like those found previously between sugars and xerophytic features [21] or dehydration-tolerance of grass species [2] support a positive role for sugars during water deficiency stress. Relatively few studies have recorded differences in sugar accumulation between individual plant varieties and, in comparisons of different species and little attention has been given to the type of sugar which increased. One of them [22] observed a correlation between glucose or fructose content and the degree of stress adaptation in cotton plants.

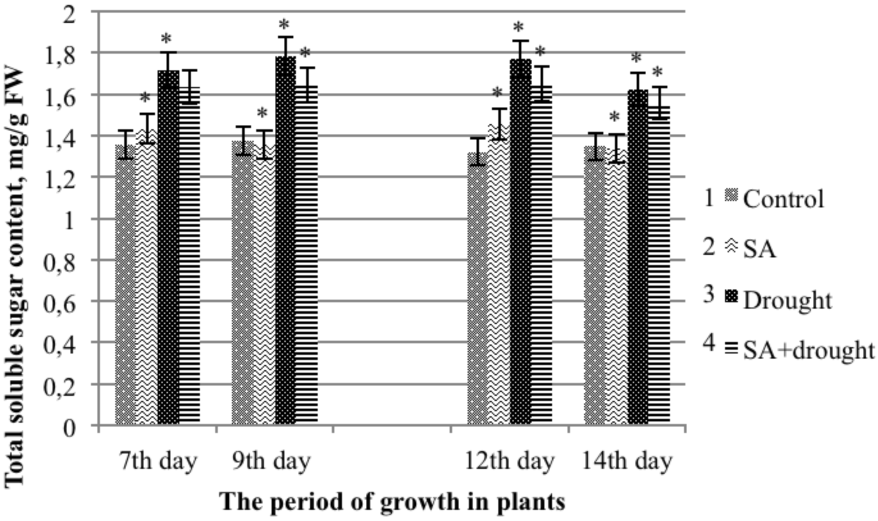

Fig. 1. The effect of salicylic acid on sugar content in the plants of wheat (Triticum aestivum L.) under drought conditions: 1 – control, 2 – salicylic acid (50μM), 3 – drought (30 %), 4 – salicylic acid (50μM) + drought (30 %). * – significant differences compared with control at p≤0,05

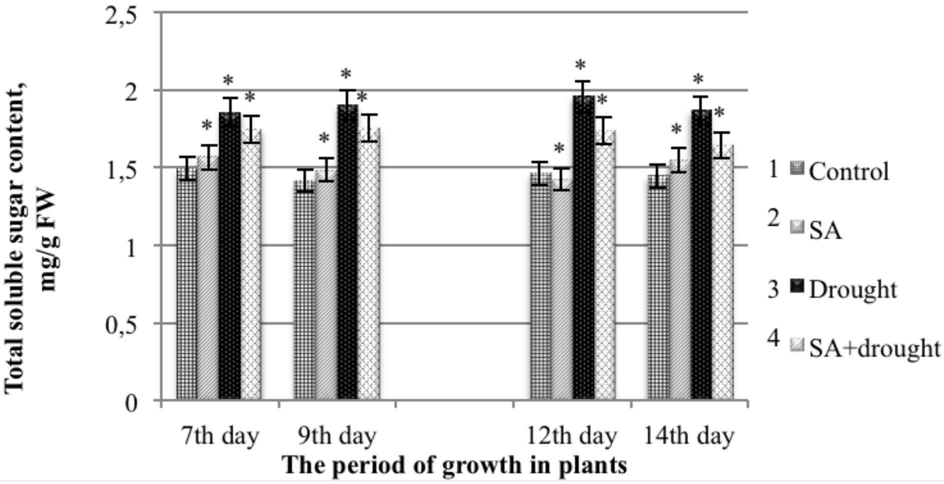

Fig. 2. The effect of salicylic acid on sugar content in the plants of corn (Zea mays L.) under drought conditions: 1 – control, 2 – salicylic acid (50μM), 3 – drought (30 %), 4 – salicylic acid (50μM) + drought (30 %). * – significant differences compared with control at p≤0,05

It was observed that both under short and long-term drought conditions wheat plants are characterized by a significant accumulation of fructose compared to plants which were not treated with SA (Fig. 3). On resuming the irrigation, the reduction of fructose content in the tissues of the control plants is more intense in plants grown from the seeds treated with salicylic acid.

Our results are coinciding with other findings [1] according to which under drought conditions salicylic acid induces accumulation of fructose in the tissues of roots and shoots of sunflower seedlings. One of the mechanisms of plant adaptation to water deficit conditions is the regulation of osmotic pressure by the accumulation of soluble compounds capable of maintaining osmotic balance between the cytoplasm and vacuole [28]. The ability to accumulate osmotically active substances such as proline, sucrose, monosaccharides, raffinose, stahiozu largely testifies about the plant's resistance to stress [11].

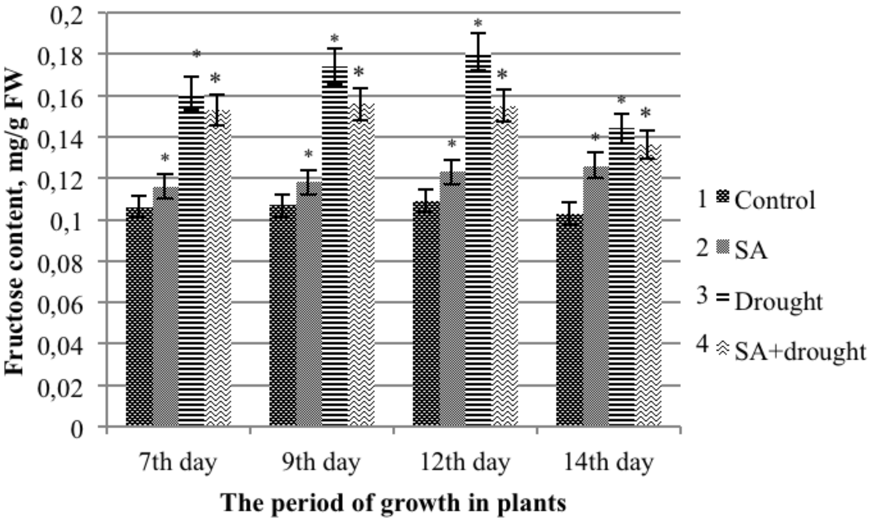

Fig. 3. The effect of salicylic acid on fructose content in the plants of wheat (Triticum aestivum L.) under drought conditions: 1 – control, 2 – salicylic acid (50µM), 3 – drought (30 %), 4 - salicylic acid (50µM) + drought (30 %). * – significant differences compared with control at p≤0,05

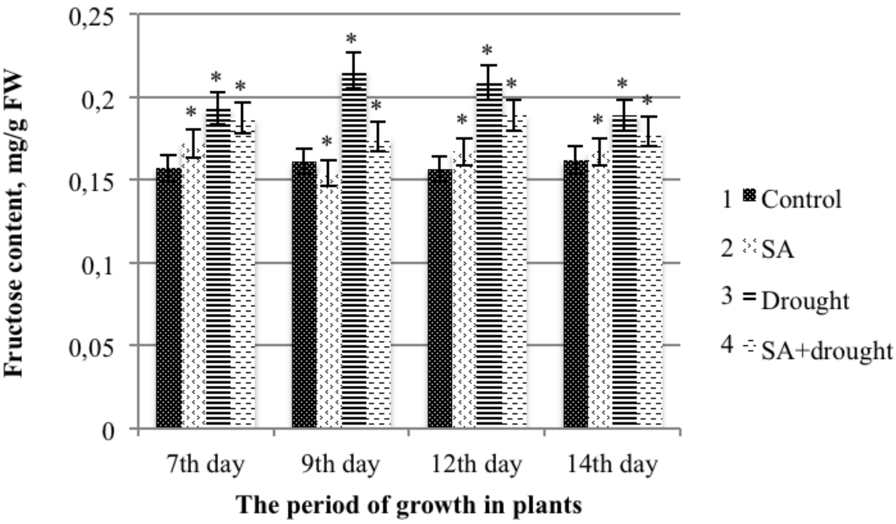

Fig. 4. The effect of salicylic acid on fructose content in the plants of corn (Zea mays L.) under drought conditions: 1 – control, 2 – salicylic acid (50µM), 3 – drought (30 %), 4 – salicylic acid (50µM) + drought (30 %). * – significant differences compared with control at p≤0,05

In the long-term drought conditions (9, 12 and one day after irrigation) we observed in the plants of corn which were treated by SA increase in fructose content compared to control (Fig. 4). The growth of this indicator for long-term drought is perhaps due to the adaptive response of plants to water shortage. Restoration of irrigation on the 14th day of growth after 1 hour initiated decrease in fructose content in corn plants to control level.

Drought stress causes different changes in the metabolism of sugar. Also, pretreatment with salicylic acid alleviates drought stress damages in the leaves of wheat. Moreover, drought stress initiates increase in fructose content in the leaves of corn and causes increase in fructose content as compared to control plants. The differences in the amount of fructose accumulation may be physiologically important and may help the plants to withstand the effects of reduced water content and to recover from it after stress is relieved.

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ВПЛИВ САЛІЦИЛОВОЇ КИСЛОТИ НА МЕТАБОЛІЗМ ВУГЛЕВОДІВ ПШЕНИЦІ ТА КУКУРУДЗИ ЗА УМОВ ПОСУХИ

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Вплив саліцилової кислоти І посухи на рослини пшениці та кукурудзи був предметом даного дослідження. Досліджували вплив посухи та саліцилової кислоти на вміст цукрів і фруктози у рослин кукурудзи (*Zea Mays* L.) сорту Жовта зубоподібна і пшениці (*Triticum aestivum* L.) сорту Подолянка. Виявлено збільшення вмісту вуглеводів у пагонах рослин кукурудзи як за короткотривалих, так і за довготривалих умов посухи. При повній вологоємності ґрунту (55–60 %) вміст фруктози У тканинах рослин кукурудзи, насіння яких було попередньо оброблено розчином саліцилової кислоти, є вищим, ніж у контролі, але значно нижчим, ніж у рослин, що зростали в умовах посухи. Попередня обробка насіння кукурудзи розчином саліцилової кислоти (50 мМ) підвищує загальний вміст вуглеводів. Саліцилова кислота підвищує загальний вміст вуглеводів у рослин пшениці за умов оптимального водозабезпечення (60 % повної вологоємності). Водночас у пагонах рослин пшениці, вирощених за дії посухи із насіння, не обробленого саліциловою кислотою, вміст фруктози був достовірно вищим, ніж у контролі. Таким чином, наші результати засвідчують, що саліцилова кислота нівелює стрес, викликаний посухою.

Ключові слова: посуха, саліцилова кислота, вуглеводи, фруктоза, *Triticum aestivum* L., *Zea mays* L.