ABSTRACT

Aims: This research explored the effect of exogenous abscisic acid (ABA) treatment on sugar metabolism in tomato fruits, and provided theoretical basis for the regulation of ABA on the growth and development of tomato fruits.

Study Design: The whole tomato plant was treated by spraying with 50 mg·L⁻¹ ABA. Spraying distilled water was as the control.

Place and Duration of Study: College of Biological Science and Technology, between February 2019 and March 2020.

Methodology: The contents of soluble sugar in tomato fruits were measured with HPLC (High performance liquid chromatography). The enzyme extraction and activities were followed the biochemical method.

Results: Exogenous ABA treatment reduced the weight and transverse meridians of the fruit at the ripening stage, but increased the contents of fructose, glucose and sucrose in the fruit. At the same
time, it also increased the activities of acid invertase, neutral invertase, and sucrose phosphate synthase at the ripening stage.

**Conclusions:** Exogenous ABA treatment was beneficial to the accumulation of sugar in tomato fruit and improved tomato quality. Therefore, it could be applied to tomato production.

**Keywords:** Tomato fruits; ABA; soluble sugar; sugar metabolism related enzymes.

## 1. INTRODUCTION

Fruit sugar metabolism is an important material basis for the formation of tomato (*Solanum lycopersicum*) quality, and the key to controlling the accumulation of sugar in tomato fruits is the decomposition of sucrose inside the fruit [1]. Sucrose metabolism is an important part of fruit sugar accumulation. The key enzymes are acid invertase (AI), neutral invertase (NI), sucrose synthase (SS) and sucrose phosphate synthase (SPS) involved in the synthesis and decomposition of sucrose [2,3]. Sugar metabolism in fruits and changes in related enzyme activities are important aspects of the regulation of fruit development and are closely related to quality formation [4]. When Wang Yong-zhang [5] studies the relationship between sugar metabolism and enzymes in Red Fuji apple fruit, he finds that sucrose content is mainly regulated by SS, while fructose and glucose are regulated by AI.

During fruit development, sucrose metabolism is regulated by hormones [6-10]. Abscisic acid(ABA) is a sesquiterpene plant hormone with isoprene as the basic structural unit [11]. In the different stages of fruit development, the accumulation of sugar is promoted to a certain extent by ABA. ABA mainly regulates fruit sugar metabolism by affecting the activities of enzymes related to sugar metabolism, but the effect pattern of ABA in tomato on the activities of enzymes related to sugar metabolism and sugar contents in fruit is still unclear. Therefore, the changes of tomato fruit growth and development, the contents of fructose, glucose, sucrose, and sucrose metabolism related enzyme activities in different development stages were analyzed after spraying the whole plant with ABA in this research, and the effects of ABA treatment on tomato fruit sucrose metabolism were discussed, in order to lay a foundation for further regulating tomato sugar accumulation and quality through plant growth regulators.

## 2. MATERIALS AND METHODS

### 2.1 Materials

‘Liaoyuan Duoli’ is a tomato variety bred from the Liaoning Academy of Agricultural Sciences. The tomato seeds with consistent germination were planted in nutrient by soaking seeds and acceleration germination. When the tomato plants had grown to the appropriate size, selected the same growth of the plants and transplanted them into the greenhouse with a row spacing of 35cm and a plant spacing of 50 cm.

The whole tomato plants were sprayed with 50 mg·L⁻¹ of ABA at 14d, 34d and 54d after anthesis, and distilled water was sprayed as a control at the same time. Then, after 6 d of treatment, that is, 20d, 40d, and 60d after the tomato anthesis, samples were taken, and each sample was repeated three times for the determination of sugar contents and enzyme activities.

### 2.2 Determination of Fruit Weight and Vertical and Horizontal Diameter

The mass of single fruit was measured by one percent analytical balance, the longitudinal and transverse diameters of tomato fruits were measured by electronic digital display caliper.

### 2.3 Determination of Sugar Content

The sugar content was measured with HPLC (High performance liquid chromatography): weighing about 1 g fresh weight sample→pouring 80% ethanol→bathing at 80°C for 1 h→pouring ethanol extract into 25 mL volumetric flask→adding 80% ethanol to the remaining sample and bathing at 80°C for 1 h, repeating extraction twice→pouring into volumetric flask, constant volume to 25 mL→concentrating and dissolving in 1 mL ultrapure water→supernatant through 0.45 μm filter membrane.
The determination chromatographic conditions were amino column, column temperature 30℃, Agilent 1100 differential detector, mobile phase ratio 80% acetonitrile, 20% ultrapure water, flow rate of 1.0 mL·min⁻¹.

2.4 Extraction and Activity Determination of Enzymes Related to Sugar Metabolism

The enzyme extraction followed the method of Wang Yong-zhang and Zhang Da-peng [12]. Approximately 1 g fresh weight of frozen tomato tissue was ground in extraction buffer [50 mmol·L⁻¹ Hepes (pH7.5), 1 mmol·L⁻¹ EDTA, 10 mmol·L⁻¹ MgCl₂, 2.5 mmol·L⁻¹ DTT, 10 mmol·L⁻¹ ascorbate]. Nought point five gram of insoluble polyvinylpolypyrrolidone (PVPP) and three times sample volume buffer were used for each extraction. Homogenates were centrifuged at 12 000×g for 20 min at 4°C and the pellets were discarded. Ammonia sulphate was gradually added to the supernatant to 80% saturation and the solution was again centrifuged at 12 000×g for 30 min at 4°C. The supernatant was discarded and the pellet was dissolved in 2-5 mL of extraction buffer, then dialyzed against a ten-fold volume of extraction buffer for 20 h. All steps were carried out at 0-4°C.

The enzyme activity determination followed the method by Yu Xin-jian with minor modification [13]. The activities of enzymes related to sucrose metabolism (acid invertase, neutral invertase, sucrose synthase and sucrose phosphate synthase) were assayed in desalted extract.

3. RESULTS AND ANALYSIS

3.1 Effects of Exogenous ABA Treatment on Tomato Fruit Growth at Different Development Stages

As shown in Fig. 1, in the young fruit stage and expansion stage of tomato, the weight and transverse diameter of the fruit were significantly higher than those of the control after ABA treatment, while in the process of gradual ripening of tomato fruit, the weight of the fruit was lighter than that of the control, which was 38.71% less than the control. And the transverse diameter of the fruit was also lower than that of the control, which was 16.37% less than that of the control. But the longitudinal diameter of the fruit was increased, which was 11.60% higher than that of the control. The results showed that ABA had a regulatory effect on the growth and development of tomato fruits.

3.2 Effects of ABA Treatment on Soluble Sugar Contents of Tomato Fruit at Different Development Stages

As shown in Fig. 2, in the young fruit stage and expansion stage of tomato, ABA treatment decreased the contents of fructose, glucose and sucrose, but increased the contents of fructose and glucose in the fruit maturity stage. Nevertheless, the sucrose content was increased in the whole fruit growth and development period. When the tomato fruit was mature, the contents of fructose, glucose and sucrose in tomato fruit treated with ABA were 1.28, 1.20 and 1.37 times higher than those of the control, respectively. The results showed that ABA treatment could increase the contents of fructose and glucose during fruit ripening. Although the sucrose content decreased as the fruit matured, the ABA treatment group was higher than that of the control. Thus, the ABA treatment could increase the soluble sugar contents in the ripe tomato fruits.

3.3 Effects of ABA Treatment on Activities of Enzymes Related to Sugar Metabolism in Tomato Fruits at Different Developmental Stages

As shown in Fig. 3, in the young fruit stage and expansion stage of tomato, the activities of AI and NI of the ABA treatment group were lower than those of the control group, but higher than those of the control group at fruit maturity stage. The activity of SS was lower than that of the control group at fruit young stage, and higher than the control group in the fruit expansion stage and maturity stage. The activity of SPS was higher than the control group at the early stage of fruit development and maturity, and lower than the control group at the fruit expansion stage. The results showed that ABA mainly increased the soluble sugar contents in mature tomato fruits by increasing the activities of AI, NI and SPS.

4. DISCUSSION

The accumulation of soluble sugar is the basic raw material for the formation of fruit quality, which directly affects the edible value of the fruit [14]. The ABA can promote the accumulation of sugar in fleshy fruits at different developmental stages to a certain extent, and it can promote the
absorption of sugar in grape fruits from the slow growth stage to the ripening stage [15]. ABA treatment with 10 mg·L−1 significantly increased the contents of glucose, fructose and total sugar in ‘Cara cara’ Navel Orange fruit during ripening [16]. The soluble sugar content of strawberries increased after ABA treatment [17].

ABA promoted the strength of plant sink, which is related to ABA promoting the unloading of assimilates from phloem, the absorption of assimilates by sink cells and the metabolic transformation of assimilates in sink cells [18]. Exogenous ABA promotes the transport of more photosynthetic products to fruit and increases the contents of soluble sugar in mature fruit by enhancing the strength of apple fruit bank [19]. In addition to promoting the absorption of assimilates and facilitating the accumulation of assimilates in sink tissue, ABA can also prevent the outflow of assimilates through the plasma membrane of sink cells. ABA with 10-5 mg·L−1 can limit the release of various sugar components from strawberry fruit slices [20]. In this study, exogenous ABA treatment promoted the accumulations of fructose, glucose and sucrose in mature tomato fruits. It might be that exogenous ABA treatment promoted the accumulation of sugar in fruit and inhibited the outward release of sugar. The activities of sugar metabolizing enzymes during fruit ripening is crucial to the accumulation of sugar in the fruit. Grape fruit by bagging affects the sucrose metabolism of fruit, thus reduces the content of total soluble sugar. The reason may be that bagging of fruit affects the activities of sucrose metabolic enzymes [21]. Foliar spraying of selenium can increase the activity of acid invertase and promote fruit sugar metabolism [22]. ABA can also promote the conversion of sucrose by regulating the activity of invertase. ABA affects the activities of many sucrose metabolism related proteases during grape fruit ripening, and the activities of SS, cwINV and vINV are increased [23]. Proteomics is used to study grape fruit and found that ABA has an effect on the activities of many proteases related to glucose metabolism during fruit ripening, such as GIN1 and ME [24].

![Fig. 1. Effects of exogenous ABA treatment on fruit weight and longitudinal, transverse diameter of tomato](image1)

![Fig. 2. Effect of ABA treatment on soluble sugar contents of tomato fruit](image2)
In this study, exogenous ABA treatment increased the activities of AI, NI and SPS of sucrose related metabolic enzymes in tomato fruit, which might be one of the reasons for promoting the accumulation of soluble sugar in tomato fruit, but the mechanism of ABA affected enzyme activities and thus the contents of soluble sugar needed further study. Therefore, according to the results of this study, we speculated that ABA might be the initiating factor of fruit sugar accumulation, and activated by promoting the activities of enzymes related to sucrose metabolism [25]. With the continuous improvement of people’s requirements for fruit quality, the sugar contents of the fruit can be effectively improved through the use of various control technologies, and the improvement of fruit quality has become a research hotspot. In the future research, the regulation mechanism combined with the regulation technology of sugar metabolism, provide a feasible way to improve fruit quality, and can be used in the actual production processes.

5. CONCLUSION

After exogenous ABA treatment of tomato plants, ABA had a regulatory effect on the growth and development of tomato fruits. In terms of nutritional quality, ABA promoted the accumulations of fructose, glucose and sucrose in the fruit, and at the same time improved the activities of AI, NI and SPS during the ripening period of the fruit. This study provided a theoretical basis for using exogenous ABA to regulate tomato quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Offer CE, Patrick JW. Cellular pathway and hormonal control of short-distance transfer in sink regions. Plant Biology. 1986;1:295-306.
2. Islam S. Sucrose metabolism in domesticated cherry tomato, Lycopersicon esculentum var. cerasiforme Alef., and purification of sucrose synthase. The Journal of Horticultural Science and Biotechnology. 2001;76(1):40-47.
3. Keutgen AJ, Pawelzik E. Impacts of NaCl stress on plant growth and mineral nutrientassimilation in two cultivars of strawberry, Environmental and Experimental Botany. 2008;65(2):170-176.
4. Dong ZJ, Yu YY, Li SH, Wang J, Tang SJ, Huang RF. Abscisic acid antagonizes ethylene production through the ABI4-Mediated transcriptional repression of ACS4 and ACS8 in Arabidopsis. Molecular Plant. 2016;9(1):126-135.
5. Wang YZ, Zhang DP. A study on the relationship between acid invertase, sucrose synthase and sucrose metabolism in red ‘Fuji’ apple fruit. Acta Horticulturae Sinica. 2001;28(3):259-261.
6. Duan N, Jia YK, Xu J, Chen HL, Sun P. Research progress on plant endogenous hormones. Chinese Agricultural Science Bulletin. 2015;31(2):159-165.
7. Obroucheva NV. Hormonal regulation during plant fruit development. Russian Journal of Development Biology. 2014;45(1):11-21.
8. Pieterse C, Leon-Reyes A, Ent S, Wees S. Networking by small-molecule hormones in plant immunity. Nature Chemical Biology. 2009;5(5):308-316.
9. Szantner A, Calderon-Villalobos L, Estelle M. Plant hormones are versatile chemical regulators of plant growth. Nature Chemical Biology. 2009;5(5):301-307.
10. Chen S, Wang XJ, Tan GF, Zhou WQ, Wang GL. Gibberellin and the plant growth retardant Paclobutrazol altered fruit shape and ripening in tomato. Protoplasma. 2020;257(3):853-861.
11. Hauser F, Waadt R, Schroeder JI. Evolution of abscisic acid synthesis and signaling mechanisms. Current Biology. 2011;21(9):R346-R355.
12. Wang YZ, Zhang DP. Regulating effects of ethylene on carbohydrate metabolism in 'Starkrimson' apple fruit during the ripening period. Acta Horticulturae Sinica. 2000;27(6):391-395.
13. Yu XJ. Handbook of plant physiology experiments. Shanghai Science and Technology Press. 1985;148-149.
14. Chen M, Jiang Q, Yin XR, Lin Q, Chen JY, Allan AC, Xu CJ, Chen KS. Effect of hot air treatment on organic acid-and sugar-metabolism in Ponkan (Citrus reticulata) fruit. Scientia Horticulturae. 2012;147:118-125.
15. Wang L, Brouard E, Hilbert G, Renaud C, Petit JP, Edwards E, et al. Differential response of the accumulation of primary and secondary metabolites to leaf-to-fruit ratio and exogenous abscisic acid. Australian Journal of Grape and Wine Research. 2021;27(4):527-539.
16. Wang GY, Xia RX, Zeng XG, Wu QS. Dynamic changes of sugar concentrations in pulp of 'Cara cara' Navel Orange (Citrus sinensis L. Osbeck) after application by exogenous ABA and GA3. Agricultural Science & Technology. 2014;15(1):47-51.
17. Jia HF, Chai YM, Li CL, Lu D, Luo JJ, Qin L, Shen YY. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiology. 2011;157(1):188-199.
18. Zhou R, Yang HQ, Shu HR. Regulation of plant sink strength by abscisic acid. Plant Physiology Communication. 1996;3(3):223-228.
19. Sha JC, Jia ZH, Zhang X, Wu XX, Ge SF, Jiang YM. Effects of exogenous ABA on translocation of photosynthate to fruit of Fuji apple during late stage of fruit rapid-swelling. Chinese Journal of Applied Ecology. 2019;30(6):1854-1860.
20. Ofosu-Anim J, Yamaki S. Sugar content and compartmentation in melon fruit and the restriction of sugar efflux from flesh tissue by ABA. J Japan Soc Hort Sci. 1994;63(3):685-692.
21. Zha Q, Xi XJ, He YN, Fang XP, Jiang AL. The effect of bagging on the quality of
table grape ‘Hupei 1’. Sino-overseas Grape-vine Wine. 2019;2(2):54-57.

22. Zhu SM, Liang YL, An XJ, Kong FC, Gao DK, Yin HF. Changes in sugar content and related enzyme activities in table grape (Vitis Vinifera L.) in response to foliar selenium fertilizer. J Sci Food Agric. 2017;97(12):4094-4102.

23. Murcia G, Pontin M, Piccoli P. Role of ABA and gibberellin A3 on gene expression pattern of sugar transporters and invertases in Vitis vinifera cv. Malbec during berry ripening. Plant Growth Regul. 2018;84(2):275-283.

24. Giribaldi M, Geny L, Delrot S, Schubert A. Proteomic analysis of the effects of ABA treatments on ripening Vitis vinifera berries. J Exp Bot. 2010;61(9):2447-2458.

25. Trouverie J, Thévenot C, Rocher JP, Sotta B, Prioul JL. The role of abscisic acid in the response of a specific vacuolar invertase to water stress in the adult maize leaf. J Exp Bot. 2003;54:2177-2186.

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