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Article

Gene expression in some maize genotypes

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ABSTRACT

Three genotypes of maize, namely (Baghdad, 5018, and Sara) were investigated in the fields of the College of Agricultural Engineering Sciences, University of Baghdad/ Al-Jadriya, in the fall season 2021-2022. With three sowing dates (26 July, 4 August, 12 August), The experiment was implemented according to a randomized complete block design (RCBD) with three replications. The aim is to detect the expression of the INCW1 gene associated with sucrose-to-glucose analysis, which is critical for many metabolic functions in different sink tissues, and determine the amount of fold gene expression to find out which of the genotypes contain the invertase gene, and which ones have a high level of gene expression based on the fold of gene expression. The results showed significant differences in the invertase enzyme activity for the genotypes. The best genotype was (Sara) which showed a value of 1.14 mg-1.units.protein—followed genotype (Baghdad) 0.86 mg-1.units.protein. The least effective protein was for genotype (5018), which showed a value of 0.40 mg-1.units.protein. As for the sowing dates, the second sowing date (D2) was the best, which showed a value of 1.39 mg-1.units.protein. Followed by the third sowing date (D3), which showed a value of 0.51 mg-1.units.protein, and the first sowing date (D1), which showed the most negligible value of 0.51 mg-1units. The expression of the INCW1 gene varied in the genotypes of three sowing dates with values ranging between (0.30) and (2.38), where the genotype (Sara) at the second sowing date (D2), which showed the value highest of gene expression (2.38) and the exact date (D3) gave INCW1 gene showed value (2.21) for genotype (Baghdad). The genotypes also varied in their tolerance to increase or decrease the gene expression, which is considered one of the most important sucrose decomposition genes for maize crops.

Keywords: - Invertase, gene expression, Zea mays. L, Sowing date, Genotype.

INTRODUCTION

The advent of molecular marker technology has changed plant breeding methods for the better, and the increase in the activity of enzyme invertase plays a regulatory role in the early stages of grain development and formation by converting sucrose from the leaves to hexose to support cell division in the endosperm and fetus[1].

One of the main mechanisms for increasing the accumulation of hexose sugars, which stimulate cell elongation in maize, is the activity of the invertase gene[2]. The important role of cell wall genes in plant development makes their genes a good choice for isolation [3,4]. The content of the Invertase enzyme is regulated by the protein Inhibitors (INVINTLS) formed seven days after insemination 5. Then, the development of the growth seed of maize continues through regular stages after fertilization. The endosperm and seed are completely dependent on photosynthesis in the leaves. The Sac-hydrolyzing enzyme that produces Hexose Sugar from sucrose is necessary for the developmental stages of maize grain formation and composition, which determines the plant's yield stability.

Invertase, separately or integrated with plant hormones, can regulate many plant growth and development traits, as sucrose plays a major role in carbon metabolism, and carbohydrates are synthesized in the leaves (source) as sucrose, originated and transported to (sink) [7]. In maize, Ivr2 and Ivr1 gene expression is enhanced in the presence and absence of polysaccharides[8, 9]. Transported sucrose provides energy and carbon for plant growth and development and regulates gene expression [6].

The Invertase enzyme of the INCW1 gene is one of the enzymes that introduce carbon into the process of carbon metabolism, where carbon metabolism is a hallmark of C3 plants, including maize, and sugars control the invertase gene INCW1 in an orderly manner [10].

The function of the INCW1 gene is to transport carbohydrates into pollen[11]. Two genes encoding sugars in the invertase enzyme Ivr2, and Ivr1 both develop calories and pollen but their gene expression pattern is different, Ivr1 is upregulated by removing carbohydrates, while Ivr2 is by increasing the supply of carbohydrates [4, 12]

INCW1 gene appears in two forms, Incw1-S (small) and Incw1-L (large), since both metabolizable and non-metabolizable sugars are present in Incw1-L[13]. The INCW1 gene is one of the enzymes that introduce carbon into the metabolism process, where carbon metabolism is a hallmark of C3 plants, including maize, and sugars control the invertase gene Incw1 in an orderly manner [9].

The cell wall invertase gene Incw1 located in the endosperm maintained its effect on the seed weight of maize plants [14, 15']. The Incw1 gene may affect the weight of 100 kernels by controlling the growth and development of the maize cob [16]. INCW1 gene levels are increased in old and whole leaves but decreased in young maize leaves [17].

The mechanism of action of the invertase enzyme:-

Stimulation of pathways enzyme, which is known for sucrose splitting in plants, is carried out by two pathways, the first by invertase

(sucrose + (H2O) $\Rightarrow$ glucose + fructose)

Moreover, the second is the enzyme sucrose (SUS).

(Sucrose (SUS) + UDP $\Leftrightarrow$ Fructose + glucose UDP)

Both pathways work on the decomposition of sucrose in plants, but the products of their reactions differ.

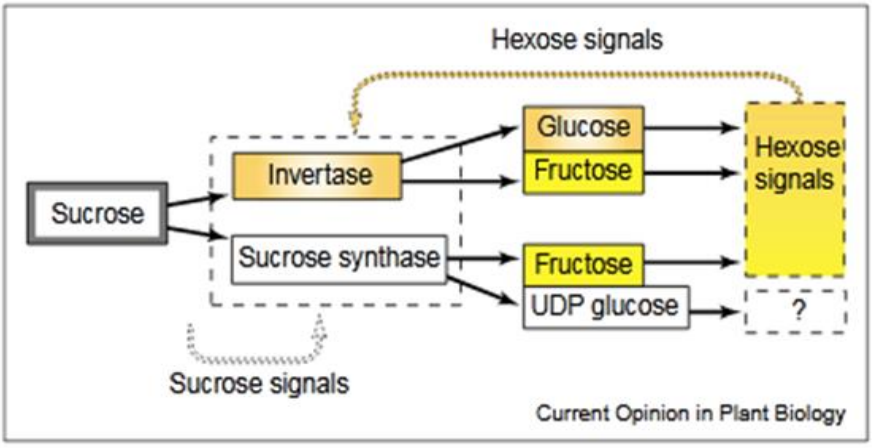

Figure 1.The mechanism of action of the invertase enzyme [18].

It produces invertase (glucose) instead of (glucose UDP) and thus forms a double number of hexose sugars such as (glucose and fructose). The resulting signals can change the expression of various genes, so invertase can be a powerful influencer in the biosynthesis and knowledge of hormones such as abscisic acid (ABA). In addition, glucose and hormone signals can also influence the expression of genes that encode sucrose synthase and invertase.

MATERIALS AND METHODS

Three genotypes of maize were used in the field Experiment, which are Baghdad, 5018, Sare Var., where Invertase activity was estimated by adding a stock solution that was prepared from (DNSA reagent, NaoH, Roshan's salt, starch and sucrose). At a ratio of 2000 µl to 10 µl, the sample powder is put in a water bath for 20 minutes at a temperature of 37 ° C, then placed in a Spectrophotometer at a wavelength of 540. According to [19].

Gene Expression

RNA extraction

The samples (leaves) were collected and immersed in triol solution at room temperature for 24 hours, whereby RNA was extracted from the sample according to the TRI Reagent method (Molecular Research Center, Cincinnati, OH, USA) [20]

Real-time quantitative PCR:

The quantification of pure gene mRNA was performed by Real-time quantitative PCR. Use SYBR Green dot dye to quantify cDNA using Mastermix as recommended by the manufacturer.

Gene expression analysis using the Levac method:

ΔCT = CT gene - CT House Keeping gene

$\Delta\Delta CT$ =ΔCT Treated or Control - ΔCT Control

The fold of gene expression = $2^{-\Delta\Delta CT}$

RESULTS

The results of estimating the invertase enzyme activity INV are in Table.1 indicates the superiority of the genotype Sara (V3) by giving the highest average invertase activity of 1.14 mg^{-1} units.protein, followed genotype Baghdad (V1) 0.90 mg^{-1}.units.protein.While genotype 5018 (V2) was the least effective for invertase activity, it was 43.69 mg^{-1}.units.protein. The highest mean of enzyme activity was for the second sowing date D2 (4 August) appointment, which was 1.39 g^{-1}.units.protein. Followed the third sowing date D3 (12 August) with an enzyme activity rate of 0.51mg^{-1}.units.protein, while the first sowing date D1 (26 July) had the lowest rate of enzymatic activity, reaching 0.50 mg^{-1}.units.protein.

The interaction between genotypes and sowing dates showed that genotype Sara (V3) in the second sowing date D2 (4 August) was the best, giving an average of 2.20 mg^{-1}.units.protein. followed genotype Baghdad (V1) in the same sowing date 1.35 mg^{-1}.units.protein. Genotype 5018 (V2) gave the lowest average 0.08 mg-1.units.protein. at the third sowing date, D3 (12 August).

GENOTYPES	SOWING DATES			
	D_1	D_2	D_3	Mean genotypes
V_1	0.42	1.35	0.82	0.90
V_2	0.52	0.61	0.08	0.40
V_3	0.58	2.20	0.63	1.14
Mean sowing dates	0.50	1.39	0.51	
L.S.D $_{0.05}$ v	0.15			
L.S.D $_{0.05}$ D	0.15			
L.S.D $_{0.05}$ D×V	0.26			

Table 1. Effect of sowing dates on Invertase enzyme activity (INV) (mg-1.units.protein) genotypes of maize in fall season 2020-2021.

Table 2 Shows genotype Sara (V3), giving a gene expression value of 2.38 at the second sowing date D2 (4 August), which exceeded the genotype Baghdad (V1), which has a gene expression value of 2.21.

At the same sowing date, the difference from the comparison treatment was 1.38 and 1.28 for the Sara and Baghdad genotypes, respectively.

Genotype 5018 (V2) did not show any expression of the gene for any of the three sowing dates. The third sowing date, D3 (12 August), did not express any gene for any of the three genotypes.

Group	Sample	ACT	IVR1	DCT	DDCT	Folding
D1	(V1)	37.02	30.45	-6.57	0.00	1.00
	(V2)	35.98	28.51	-7.47	0.00	1.00
	(V3)	35.98	28.47	-7.51	0.00	1.00
	SE					
D2	(V1)	35.90	28.19	-7.71	-1.14	2.21
	(V2)	35.35	28.78	-6.57	0.90	0.54
	(V3)	35.87	27.11	-8.76	-1.25	2.38
	SE					0.58
D3	(V1)	32.09	27.17	-4.92	1.65	0.32
	(V2)	34.90	27.83	-7.07	0.40	0.76
	(V3)	34.02	28.24	-5.78	1.73	0.30
	SE					0.15

Table 2. Gene expression for three genotypes of maize under three sowing dates in the fall season 2020-2021.

DISCUSSION

The cell wall invertase gene INCW1 is essential in transferring carbohydrates from the source (leaves) to the sink (grains) [21]. The gene also has a role in grain filling and improving its productivity, and whether the increase in the gene expression of invertase enzyme will affect the growth and development stages of maize plant[22].

Heat stress reduces INV activity in maize, preventing sucrose decomposition to hexose and decreasing starch biosynthesis in the endosperm [23.] The gene expression of the invertase genes is still poorly understood [24]. Furthermore, The increase in antioxidant gene expression under high temperatures is described in [25]

CONCLUSIONS

The genotypes of maize that interact With three sowing dates (26 July, 4 August, 12 August) showed significant differences in the activity of the invertase enzyme for the genotypes. The best genotype was (Sara) which showed a value of 1.14 mg-1.units.protein. As for the sowing dates, the second sowing date (D2) was the best, which showed a value of 1.39 mg-1.units.protein. The expression of the INCW1 gene varied in the genotypes of three sowing dates with values ranging between (0.30) and (2.38), where the genotype (Sara) at the second sowing date (D2), which showed the value highest of gene expression (2.38) and the same date (D3) gave INCW1 gene showed value (2.21) for genotype (Baghdad). The genotypes also varied in their degree of tolerance to increase or decrease the gene expression, which is considered one of the most important sucrose decomposition genes for maize crops.

References

1. Wittich, P. E., & Vreugdenhil, D. (1998). Localization of sucrose synthase activity in developing maize kernels by in situ enzyme histochemistry. Journal of Experimental Botany, 49(324), 1163-1171.
2. Long, J. C., Zhao, W., Rashotte, A. M., Muday, G. K., and Huber, S. C. (2002). Gravity-stimulated changes in auxin and invertase gene expression in maize pulvinal cells. Plant Physiology, 128(2), 591-602.

3. Greiner, S., Weil, M., Krausgrill, S., & Rausch, T. (1995). A tobacco cDNA coding for cell-wall invertase. Plant physiology, 108(2), 825.
4. Xu J, A vigne WT , McCarty DR, Koch KE (1996) A similar dichotomy of sugar modulation and developmental ex- pression affects both paths of sucrose metabolism: evi- dence from a maize invertase gene family. The Plant Cell, 8: 1209–1220.
5. Sander, A., Krausgrill, S., Greiner, S., Weil, M., & Rausch, T. (1996). Sucrose protects cell wall invertase but not vacuolar invertase against proteinaceous inhibitors. FEBS letters, 385(3), 171-175.
6. Taliercio, E. W., Kim, J. Y., Mahé, A., Shanker, S., Choi, J., Cheng, W. H., ... and Chourey, P. S. (1999). Isolation, characterization and expression analyses of two cell wall invertase genes in maize. Journal of Plant Physiology, 155(2), 197-204.
7. Roitsch, T., and González, M. C. (2004). Function and regulation of plant invertases: sweet sensations. Trends in plant science, 9(12), 606-613.
8. Xu, J., Pemberton, G. H., Almira, E. C., McCarty, D. R., & Koch, K. E. (1995). The Ivr 1 gene for invertase in maize. Plant physiology, 108(3), 1293.
9. Cheng, W. H. (1997). Gene-expression analysis of acid invertase and sucrose-phosphate synthase in maize. University of Florida.
10. Datta, R., and Chourey, P. S. (2001). Sugar-regulated control of α-tubulin in maize cell suspension culture. Plant cell reports, 20(3), 262-266.
11. Goetz, M., Godt, D. E., Guivarc'h, A., Kahmann, U., Chriqui, D., and Roitsch, T. (2001). Induction of male sterility in plants by metabolic engineering of the carbohydrate supply. Proceedings of the National Academy of Sciences, 98(11), 6522-6527.
12. Rafique, S. (2019). Differential expression of leaf proteome of tolerant and susceptible maize (Zea mays L.) genotypes in response to multiple abiotic stresses. Biochemistry and Cell Biology, 97(5), 581-588.
13. Cheng, W. H., Taliercio, E. W., & Chourey, P. S. (1999). Sugars modulate an unusual mode of control of the cell-wall invertase gene (Incw 1) through its 3′ untranslated region in a cell suspension culture of maize. Proceedings of the National Academy of Sciences, 96(18), 10512-10517.
14. Ren, X. D., Liu, H. M., Liu, Y. H., Hu, Y. F., Zhang, J. J., and Huang, Y. B. (2015). Influence of sugars and hormones on the genes involved in sucrose metabolism in maize endosperms. Genet Mol Res, 14, 1671-1678.
15. Liu, J., Huang, J., Guo, H., Lan, L., Wang, H., Xu, Y., ... and Yan, J. (2017). The conserved and unique genetic architecture of kernel size and weight in maize and rice. Plant physiology, 175(2), 774-785.
16. Lu, X., Zhou, Z., Yuan, Z., Zhang, C., Hao, Z., Wang, Z., ... & Weng, J. (2020). Genetic dissection of the general combining ability of yield-related traits in maize. Frontiers in plant science, 11, 788.
17. Behr, M., Humbeck, K., Hause, G., Deising, H. B., and Wirsel, S. G. (2010). The hemibiotroph Colletotrichum graminicola locally induces photosynthetically active green islands but globally accelerates senescence on aging maize leaves. Molecular plant-microbe interactions, 23(7), 879-892.
18. Koch, K. (2004). Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Current opinion in plant biology, 7(3), 235-246.
19. Vitolo M. 2009. Enzymes: the catalytic proteins. In: Vitolo M (ed). Pharmaceutical Biotechnology, São Paulo; Edgard Blücher, p:203-28.
20. Chomczynski P, Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162: 156–159.
21. Ruan, Y. L., Jin, Y., Yang, Y. J., Li, G. J., and Boyer, J. S. (2010). Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. Molecular Plant, 3(6), 942-955.
22. Hütsch, B. W., Jung, S., and Schubert, S. (2015). Comparison of salt and drought-stress effects on maize growth and yield formation with regard to acid invertase activity in the kernels. Journal of agronomy and crop science, 201(5), 353-367.
23. Cheikh, N., & Jones, R. J. (1995). Heat stress effects on sink activity of developing maize kernels grown in vitro. Physiologia Plantarum, 95(1), 59-66.
24. Ren, X., & Zhang, J. (2013). Research progresses on the key enzymes involved in sucrose metabolism in maize. Carbohydrate Research, 368, 29-34

25. Singh, I., Debnath, S., Gautam, A., and Yadava, P. (2020). Characterization of contrasting genotypes reveals general physiological and molecular mechanisms of heat-stress adaptation in maize (Zea mays L.). Physiology and Molecular Biology of Plants, 26(5), 921-929.

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