Journal of Cambodian Health
Volume. 2, Issue. 1, 2024, v2103
https://doi.org/10.70243/jch2024.2103

OPEN ACCESS

Identification of Alpha-Galactosidase gene in tomato plant

Report

Sreyly Heng*, Meyhouy Hak, Dalen Som, and Sreynith Sort

Faculty of Health Sciences and Biotechnology, University of Puthisastra, Phnom Penh 12211, Cambodia

Article history
Received: 29 May 2024
Revised: 25 June 2024
Accepted: 28 June 2024
Published: 30 June 2024

Keywords
Alpha-Galactosidase
cDNA
PCR
Tomato plants
Oligosaccharides

Abstract: In recent years, Alpha-Galactosidase enzymes have been a growing interest in applying renewable resources for biorefineries; while tomatoes are widely consumed around the world and are the second most economically valuable vegetable. It contains the highest amount of vitamins and minerals of any fruit or vegetable. The project intends to isolate the α-Galactosidase genes from tomato plants, including leaves and seeds. The samples including both tomato leaves and seed were prepared and were extracted RNA respectively by Total RNA Extraction Kit-Mini-Plant. The total RNA elution was checked by the concentration by UV-Vis spectrophotometer. For cDNA synthesis, the isolated RNA was converted into complementary DNA via reverse transcription using TOYOBO Company reagents, and Actin PCR was used as a positive control. As the results, Alpha-Galactosidase is present in germinating of tomato seeds, where it is involved in the mobilization of carbohydrate reserves during and following tomato's seed germination, while for tomato leaves there is no evidence that illustrates the appearance of α-Galactosidase in tomato leaves. This result is significant for further research aiming for gene overexpression analysis for multiplying the enzyme α-Galactosidase amount for the medical application.

1. Introduction

Enzyme applications in industry have gained more attention as a result of the increased need and interest in using renewable resources for biorefineries in recent years (Ju et al., 2019). The α-Galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) catalyzes the hydrolysis of terminal α-1,6-linked galactosyl residues from a wide range of oligosaccharides and the polysaccharides. It also acts on glycoprotein, glycosphingolipids, transglycosylation reactions and synthetic substrates (Dey and Pridman, 1972). Nowadays, the beetroot sugar business, the paper and pulp industry, the soy food processing sector, and the animal feed industry are the industries where α-Galactosidase finds the most industrial use. Additionally, it helps in xenotransplantation, blood group change, and the management of Fabray's illness.

Soy milk is a popular traditional beverage in Asian countries, extensively consumed due to its affordability and convenience as a high-quality protein source. Most parts of research from the past few decades has shown a clear correlation between eating soy-based foods and their positive health impacts. Similar to cow's milk, soy milk has a balanced nutritional profile but is free of lactose, gluten, and cholesterol. It also contains beneficial phytochemical substances that are associated to improved health (Jiang et al., 2013). Those who are allergic to cow's milk can also use these plant-based beverages as an alternative (Huang et al., 2023). Almond and soybean seeds have naturally occurring oligosaccharides, with α-galacto-oligosaccharides such as verbascose, stachyose, and raffinose predominating. The high content of α-galactooligosaccharides in soybeans makes them a potential source of flatulence when

***Corresponding author**: Sreyly Heng, hsreyly@puthisastra.edu.kh

© 2024 University of Puthisastra Co., Ltd. All rights reserved.

consumed in goods. The excessive buildup of gas or air in the stomach or intestines is known as flatulence, which is one of the topics of extreme personal humiliation. There are a variety of symptoms that can occur including emotional and social nervousness, abdominal rumblings, stomach discomfort, cramps, pain, and diarrhea (Price et al., 1988). Studies have shown that verbascose, stachyose, and raffinose oligosaccharides are the main culprits of soybean flatulence. Intestinal bacteria ferments them after they evade digestion, producing an abundance of hydrogen and carbon dioxide. Raffinose, stachyose, and verbascose are able to pass through the intestinal mucosa of humans, rats, and pigs without the presence of α-Galactosidase. The microorganisms there can digest them to produce massive amounts of hydrogen and carbon dioxide (Rackis, 1981). Due to their potential to cause flatulence, oligosaccharides were perceived as unwanted components, which prompted various investigations to find ways to eliminate them from products made from soybeans and other legumes (Huang et al., 2022). Alpha-Galactosidase also has the main role in the biotechnological application in reducing oligosaccharides from soymilk to remove flatulence-causing factors for consumers (Patil et al., 2010). Tomatoes are widely consumed worldwide and rank second in terms of economic worth among vegetables. It is the best fruit or vegetable in terms of vitamin and mineral content. The tomato lends itself well to physiological and cytogenetic studies due to its ease of culture, high rate of reproduction, and genetic homogeneity brought about by autogamy (Rick, 2015). Tomato seeds have a 32% protein content, a 27% total fat content, and an 18% fiber content (Kumar et al., 2021). A-Galactosidase can be found in the embryos, micropylar, and lateral endosperm of tomato seeds after and during sprouting. Besides, a substantial amount of proteins could be found in tomato's leafy waste products. Phytochemical, phenolic, and flavonoid content are all abundant in tomato leaves. (Yu et al., 2023). However, there has not been any recorded data of the α-Galactosidase gene from tomato plants yet. This study reports on isolation of the α-Galactosidase genes from tomato plants including leaves and seeds.

2. Materials and method

2.1. Dissociation of tomato leave and tomato seed

Firstly, the fresh wild type of tomato leaves were cut from the tomato laboratory room. Then, all the leaves were frozen in liquid nitrogen and ground to tomato leaf powder that was kept as "sample 1". Secondly, the dried tomato seeds were kept in the fridge in the laboratory were sterilized with 5% of bleach for 2 min and rinsed in the water overnight. Then, the water was removed and the seeds were imbibed on two layers of No. 1 filter paper with double-deionized water and left in the dark at 25 ℃ for 96 hours to germinate. Next, the germinated seeds were ground with nitrogen to get ground tomato seed that was kept as "sample 2".

2.2. Extraction of total RNA

Each sample was extracted RNA respectively by Total RNA Extraction Kit-Mini-Plant, Cat. No. YRP50, which is the product of RBC Bioscience manufacturer. To follow this protocol, 50 mg of each sample powder was lysed in 500 μL RB Buffer with 5 μL β-mercaptoethanol by vortexing, and incubating at room temperature for 5 min. The lysate sample was applied into the filter column and centrifuge for 2 min at full speed (13 000 rpm). The 500 μL of the clarified filtrate was transferred to a new microcentrifuge tube to add with 250 μL ethanol and mix immediately by vortexing. The ethanol-added mixture was transferred to the RB column and centrifuge at full speed for 2 min. The flow-through was discarded; then, added 400 μL of RW1 Buffer and centrifuge at full speed for 1 min to discard the flow

© 2024 University of Puthisastra

through and place the RB column back in the collection tube. The RB column was added by 600 μL R-Wash Buffer (ethanol added) and centrifuge for 1 min same as the previous step. Next, the dried RB column was transferred to an RNase free microcentrifuge tube and added 50 μL of RNase to elute purified RNA. The total RNA elution was checked by the concentration by Shimadzu UV-1800 UV-Vis spectrophotometer; 115VAC (transfer approximately 1mL of 70 times diluted RNA into cuvette) and recorded the data.

2.2.1. cDNA synthesis

The extracted RNA was then synthesized by the complementary DNA using reverse transcription reagents from TOYOBO Company. The mixture of reverse transcription includes 0.5 μL of 25 pmoles/μL Random Primers, 2 μL of 5×Buffer, 1 μL of 10 mM dNTPs, 0.5 μL of 100 units/μL ReverTraAce®, 5 μL of total RNA (based on calculation of the concentration that was checked in UV-Vis), and adjust RNase free water up to total volume 10 μL. This mixture was treated in RT-PCR (Reverse Transcription Polymerase Chain Reaction) by the thermal cycle, which followed these conditions: 30 ℃ for 10 min, 42 ℃ for 60 min, then 99 ℃ for 5 min to get fulfilling cDNA synthesis.

2.2.2. Polymerase chain reaction

The conventional PCR was performed using the TOYOBO α-Galactosidase positive control. The mixture including 2 μL of 10× PCR Buffer for KOD-plus-Neo, 2 μL of 2 mM dNTPs, 1.2 μL $MgSO_4$, 2 μL of each action primer, 1 μL of 10 times diluted cDNA, 0.2 μL KOD plus Neo, and adjust RNase free water up to total volume 20 μL. This actin PCR mixture was treated in the thermal cycle following these conditions: 94 ℃ for 2 min, 98 ℃ for 10 sec, 55 ℃ for 30 sec, and 68 ℃ for 30 sec to code the gene of interest (α-Galactosidase), and repeat 40 cycles to amplify the gene of interest. After treatment in the thermal cycle, the PCR product was loaded in gel electrophoresis to see the DNA fragments of the gene of interest by loading 5 μL mixture of 10 times diluted of 0.5 μL marker with other 4.5 μL of PCR product and sterilized double distilled water.

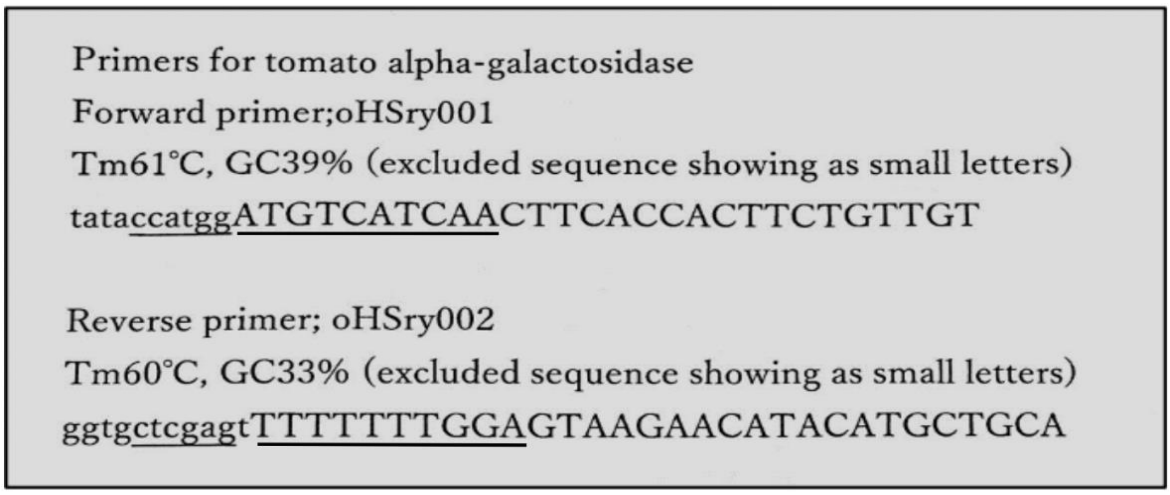

Figure 1. Primers of tomato α-Galactosidase including forward primer (oHSry001) and reverse primer (oHSry002). The underlined capital letters refer to the recognition sites of forward and reverse primers.

On the top of that, the α-Galactosidase PCR was also performed in the purpose to get the gene of interest as tomato α-Galactosidase. The used forward and reverse primers in the PCR reaction have been

© 2024 University of Puthisastra

shown in Figure. 1, while the thermal conditions were processed following: 94 ℃ for 2 min as the pre-denaturing, 98 ℃ for 10 sec as the denaturing, 55 ℃ for 30 sec as the annealing, 68 ℃ for 30 sec as the extension, and repeat for 40 cycles as the amplification.

3. Results and discussion

The mass molecular weight of tomato α-Galactosidase in gel electrophoresis was expected to show in 1.3 Kbp. As a result of the experiment, α-Galactosidase PCR product from tomato leaves and tomato seed was shown in Figure. 2 and Figure. 3. For tomato leaves, we could not get the band as the expected

Figure 2. α-Galactosidase PCR product from tomato leaves did not show any bands on the agarose gel after being treated by α-Galactosidase primers.

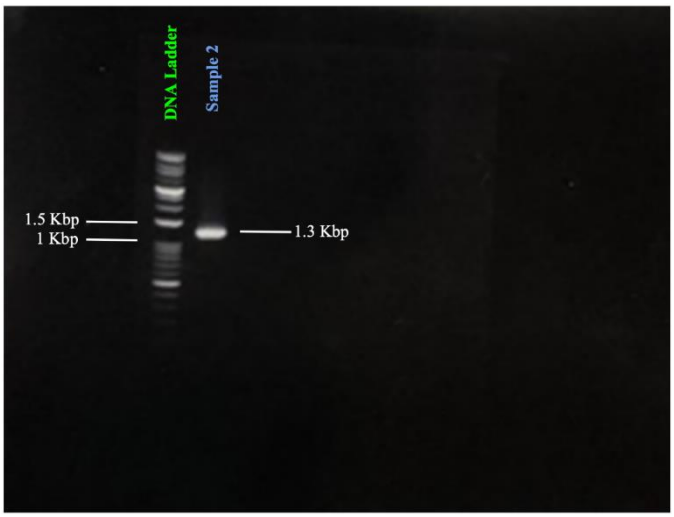

Figure 3. α-Galactosidase PCR product from tomato seed showed the target band as the expected size (1.3 Kbp)

size, though we tried to do PCR many times with many modified conditions. For tomato seed, the α-Galactosidase gene has been detected after gel electrophoresis and PCR reaction with the target molecular size 1.3 Kbp. Enzymes α-Galactosidase in plants are involved in desiccation tolerance during seed development and serve as a source of stored energy used by germinating seeds (Arunraj et al., 2020). Alpha-Galactosidase is present in germinating seeds, where it is involved in the mobilization of

© 2024 University of Puthisastra

carbohydrate reserves during and following tomato's seed germination (Bassel et al., 2001). While for tomato leaves, there is no evidence that illustrates the appearance of α-Galactosidase in tomato leaves. The tomato leaves do not need this enzyme because their main functions are gas exchange and photosynthesis. Rather, leaves concentrate on the synthesis and metabolism. Alpha-Galactosidase production in leaves would be excessive and energy wasting. Plants have evolved to express certain enzymes selectively in the tissues where they are functionally necessary, thus optimizing their metabolic processes. By preserving energy and resources, this specialization promotes effective growth and development. Thus, this highly controlled, adaptive gene expression is the cause of the lack of alpha-Galactosidase in tomato leaves (Petrović et al., 2021).

4. Conclusion

All things considered into 2 main points are tomato seed contains α-Galactosidase gene, whereas tomato leaves do not contain this gene. The α-Galactosidase gene is absent from tomato leaves due to the enzyme it encodes is not required for the metabolic activities taking place in the leaves. Rather, the gene is expressed in tissues where it is necessary for it to perform its activity, as in seeds where it aids in the release of stored carbohydrates. During the experiment, the seed was imbibed for 4 days to induce the germination of embryo and endosperm that is literally involved in the result above. In this study, tomato-seed α-Galactosidase was effectively detected and identified using specific α-Galactosidase PCR. The specific activity of the enzyme and its potential significance in tomato seed germination and development are highlighted by our findings. The identification of α-Galactosidase from tomato seeds represent a major advancement for applications in the medical field. This research sets the stage for future genetic engineering efforts aimed at overexpressing this enzyme, with promising implications for medical therapies and enhanced nutritional applications.

Author contributions

Conceptualization, methodology, formal analysis, investigation, Heng. S.; writing—review and editing, Hak. M.; Som. D. and Sort. S.

Acknowledgment

We would like to express our gratitude to the PEACE Exchange program for providing the financial support and to the supreme power of the Hiroshima University for the kind permission that allowed us to complete our research there.

Conflicts of interest

The authors express no potential conflicts of interest.

References

Arunraj, R., Skori, L., Kumar, A., Hickerson, N. M. N., Shoma, N., M., V., & Samuel, M. A. (2020). Spatial regulation of alpha-Galactosidase activity and its influence on raffinose family oligosaccharides during seed maturation and germination in Cicer arietinum. *Plant Signaling & Behavior*, *15*(8), 1709707. https://doi.org/10.1080/15592324.2019.1709707

Bassel, G. W., Mullen, R. T., & J Derek Bewley. (2001). α-Galactosidase is synthesized in tomato seeds during development and is localized in the protein storage vacuoles. Canadian Journal of Botany, 79(12), 1417–1424. https://doi.org/10.1139/b01-122

© 2024 University of Puthisastra

Bewley, J. D., & Black, M. (1978). Physiology and Biochemistry of Seeds in Relation to Germination. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-66668-1

de Vries, R. P., van den Broeck, H. C., Dekkers, E., Manzanares, P., de Graaff, L. H., & Visser, J. (1999). Differential Expression of Three α-Galactosidase Genes and a Single β-Galactosidase Gene from Aspergillus niger. Applied and Environmental Microbiology, 65(6), 2453–2460. https://doi.org/10.1128/aem.65.6.2453-2460.1999

Feurtado, J. A., Banik, M., & Bewley, J. D. (2001). The cloning and characterization of alpha-Galactosidase present during and following germination of tomato (Lycopersicon esculentum Mill.) seed. Journal of Experimental Botany, 52(359), 1239–1249. https://pubmed.ncbi.nlm.nih.gov/11432942/

Huang, Y.-P., Paviani, B., Fukagawa, N. K., Phillips, K. M., & Barile, D. (2022). Comprehensive oligosaccharide profiling of commercial almond milk, soymilk, and soy flour. Food Chemistry, 135267. https://doi.org/10.1016/j.foodchem.2022.135267

Jiang, S., Cai, W., & Xu, B. (2013). Food Quality Improvement of Soy Milk Made from Short-Time Germinated Soybeans. Foods, 2(2), 198–212. https://doi.org/10.3390/foods2020198

Ju, L.-K., Loman, A. A., & Mahfuzul Islam, S. M. (2019, January 1). α-Galactosidase and Its Applications in Food Processing (L. Melton, F. Shahidi, & P. Varelis, Eds.). ScienceDirect; Academic Press. https://www.sciencedirect.com/science/article/abs/pii/B9780081005965216430?via%3Dihub

Kumar, M., Tomar, M., Bhuyan, D. J., Punia, S., Grasso, S., Sá, A. G. A., Carciofi, B. A. M., Arrutia, F., Changan, S., Radha, Singh, S., Dhumal, S., Senapathy, M., Satankar, V., Anitha, T., Sharma, A., Pandiselvam, R., Amarowicz, R., & Mekhemar, M. (2021). Tomato (Solanum lycopersicum L.) seed: A review on bioactives and biomedical activities. Biomedicine & Pharmacotherapy, 142, 112018. https://doi.org/10.1016/j.biopha.2021.112018

Leung, D. W. M., & Bewley, J. D. (1983). A role for α-Galactosidase in the degradation of the endosperm cell walls of lettuce seeds, cv. Grand Rapids. Planta, 157(3), 274–277. https://doi.org/10.1007/bf00405194

Patil, A. G. G. (2010). α-Galactosidase from Bacillus megaterium VHM1 and Its Application in Removal of Flatulence-Causing Factors from Soymilk. Journal of Microbiology and Biotechnology, 20(11), 1546–1554. https://doi.org/10.4014/jmb.0912.12012

Petrović, I., Savić, S., Gricourt, J., Causse, M., Jovanović, Z., Stikić, R. (2021). Effect of long-term drought on tomato leaves: the impact on metabolic and antioxidative response. Physiol Mol Biol Plants, (12):2805-2817. https://doi.org/10.1007%2Fs12298-021-01102-2

Price, K. R., Lewis, J., Wyatt, G. M., & Fenwick, G. R. (1988). Review article Flatulence — Causes, relation to diet and remedies. Food / Nahrung, 32(6), 609–626. https://doi.org/10.1002/food.19880320626

Rackis, J. J. (1981). Flatulence caused by soya and its control through processing. Journal of the American Oil Chemists' Society, 58(3Part3), 503–509. https://doi.org/10.1007/bf02582414

Rick, C. M. (2015). Tomato. Hybridization of Crop Plants, 669–680. https://doi.org/10.2135/1980.hybridizationofcrops.c48

Yu, Y., Kleuter, M., Taghian Dinani, S., Trindade, L. M., & van der Goot, A. J. (2023). The role of plant age and leaf position on protein extraction and phenolic compounds removal from tomato (Solanum lycopersicum) leaves using food-grade solvents. Food Chemistry, 406, 135072. https://doi.org/10.1016/j.foodchem.2022.135072

© 2024 University of Puthisastra