



Identification of Gibberellins and Cytokinins in Saladette Tomato Seeds

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Authors' contributions

This research was carried out in collaboration among all authors. Authors HR, MCCR, AIMA and UAMC designed the study, wrote the protocol, managed the experimental process, analyses of the study, performed the laboratory analysis and wrote the manuscript. Authors AZG, DJC and JAVQ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide, mainly as a result of its economic and nutritive contribution to human society. The presence of endogenous gibberellins and cytokinins in seeds of several crops has been related to a good germination; however, little is known in tomato. Therefore, the aim of this study was to analyze and identify the presence of gibberellins and cytokinins in saladette tomato seeds.

Study Design: Laboratory analysis for gibberellins and cytokinins were conducted in solvent solutions groups with three technical replicates using a complete randomized design and results

when applicable were analyzed using the statistical program 'RStudio' (version 10) and data obtained subjected to a comparison of means with the Tukey ($P \leq 0.05$) test.

Place and Duration of Study: The experiment was conducted at the Department of Horticulture in Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico, during 2020-2021.

Methodology: Lots of 50 grams dry weight of saladette "SVTE8832" tomato seed samples were freeze dried and prepared with several organic solvents for the extraction, purification and identification of gibberellins and cytokinins using the techniques of gas chromatography mass spectrometry (GC-MS) and gas chromatography mass spectrometry with selection ion monitoring (GCMS-SIM) respectively.

Results: Gibberellins A₁, A₄, A₇, A₉, A₁₂, A₁₅, A₁₇, A₂₀, A₄₄ and A₅₃ were found in tomato seed tissue. The cytokinins zeatin and zeatin-ribozide were also detected in analyzed tomato samples.

Conclusion: The endogenous gibberellins A₁, A₄, A₇, A₉, A₁₂, A₁₅, A₁₇, A₂₀, A₄₄, A₅₃ and the cytokinins zeatin and zeatin-ribose are present in saladette "SVTE8832" tomato seeds.

Keywords: Tomato; seeds; gibberellins; cytokinins.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide, mainly as a result of its economic and nutritive contribution to human society. It represents the 30% of total world's cultivated horticulture land. China is the main producer and consumer of tomato, whereas USA is the principal importer of this crop. Recent statistics indicates that Mexico is the main exporter of tomato to USA and Canada at 90% and 65% rate of their total imports respectively [1]. A healthy tomato seedling depends from a high quality seed on germination capacity where endogenous hormones play an important role in this process [2]. Gibberellins and cytokinins have been related to seed germination of various crops such as peppers, lupins and apple [3]. Tomato and hot pepper seed treatments with gibberellic acid and 6-benzyl amino purine in a range of 40-70 mg/L⁻¹ have proved to increase germination percentage and seedling growth. The presence of endogenous gibberellins such as GA₄, GA₇ and GA₉ are reported in apple seeds and have been related to an improvement in germination and seedling development [4]; however, the presence of gibberellins and cytokinins in tomato seeds is less documented [4,5]. Therefore, the aim of this study was to investigate the possible presence of endogenous gibberellins and cytokinins in saladette tomato seeds.

2. MATERIALS AND METHODS

2.1 Plant Material, Site and Design

The present investigation was conducted during the period 2020-2021 at the plant physiology laboratory, Horticulture Department, Universidad

Autónoma Agraria Antonio Narro in Buenavista, Saltillo, Coahuila, México.

2.2 Extraction, Purification and Identification

2.2.1 Gibberellins

Saladette "SVTE8832" tomato seed samples (50 g dry weight) were water imbibed during 8 to 72 hours, frozen, freeze dried and grounded. The extraction and purification procedure prior to GC-MS for endogenous gibberellins analysis as reported by Ramírez et al. [6] is illustrated in Fig. 1. Purified tomato seeds were dissolved in a few drops of methanol and methylated with diazomethane. A portion of the methylated extract was dissolved in pyridine and treated with trimethylchlorosilane and hexamethyldisilazane. Aliquots were examined using a Pye 104 GLC coupled through a silicone membrane separator to an AEI MS30 dual beam mass spectrometer. Silanized glass columns (213 x 0.2 cm) were packed with 2% SE-33 on 80- 100 Gas Chrom Q. The He-flow rate was 25 mL/min and the column temperature was programmed from 180°C to 280°C at 2°/min. The MS was determined at 24eV at a source temperature of 210°C and a separator temperature of 190°C with a scan speed of 6.5 s per mass decade. The spectra were recorded by a DEC Linc 8 computer. Identification of gibberellins was conducted by comparison of KRI and MS spectra of their methyl ester trimethylsilyl ether derivatives with those of authentic samples.

2.2.2 Cytokinins

The procedure for the extraction and purification of cytokinins is presented in Fig. 2. Purified

samples were prepared and identified as previously described above using the modified technology reported by Palni et al. [7,8] and by Nandi et al. [9] The permethylated cytokinins fractions were quantified using a gas chromatograph-mass spectrometer (GC-MS, QP-5000; Shimadzu Inc., Kyoto, Japan) for selecting ion monitoring (SIM) analysis with a fused silica

capillary column (CBP1, 0.22 mm i.d. x 25 m; Shimadzu Inc., Kyoto Japan) according to Watanabe et al. [10]. Each sample was replicated three times using a completely randomized design with the statistical program 'RStudio' (version 10) and data obtained subjected to a comparison of means with the Tukey ($P \leq 0.05$) test when applicable.

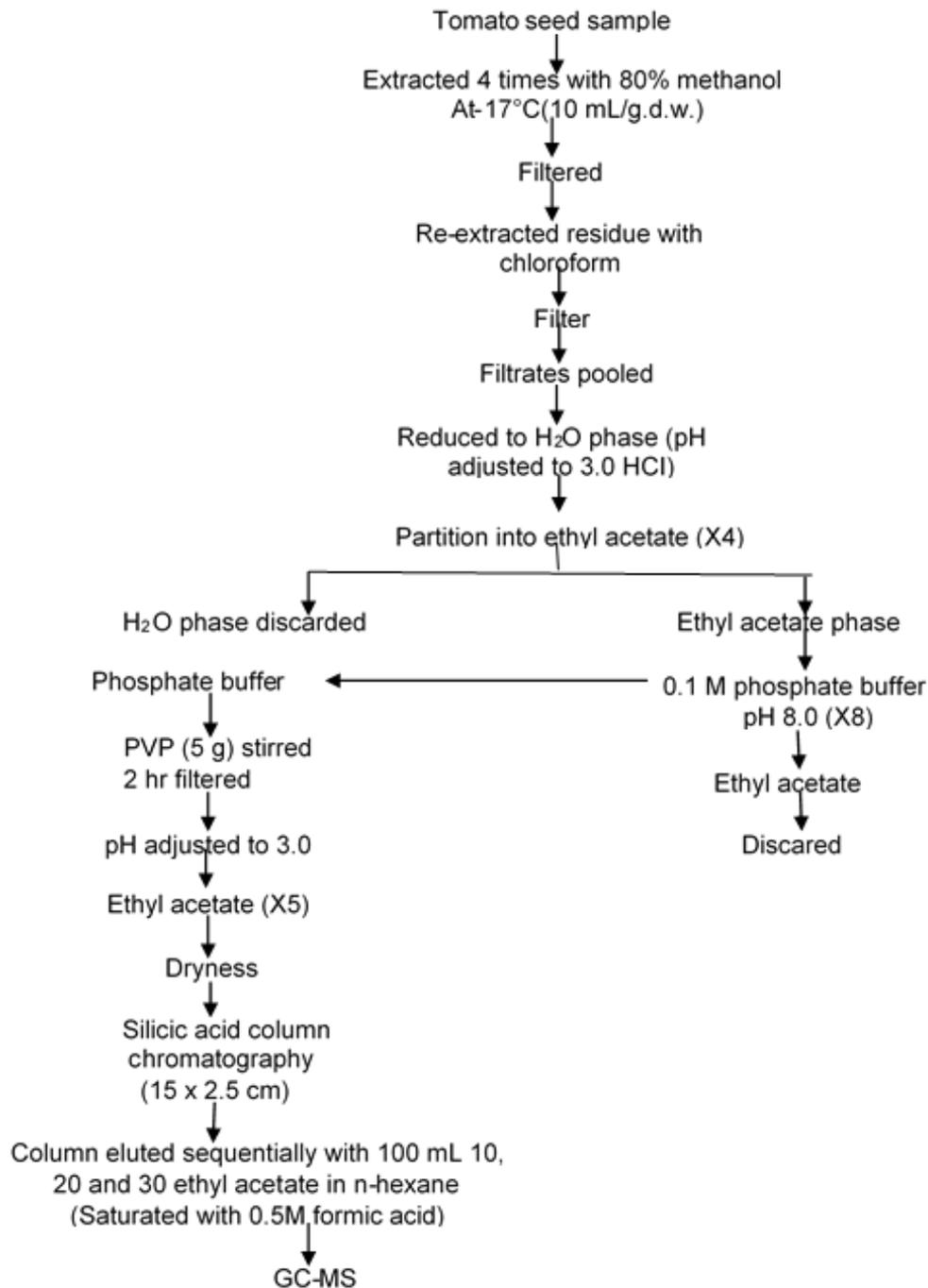


Fig. 1. Flow diagram outlining procedure used for the extraction and purification of seed extracts for gibberellin analysis using GC-MS

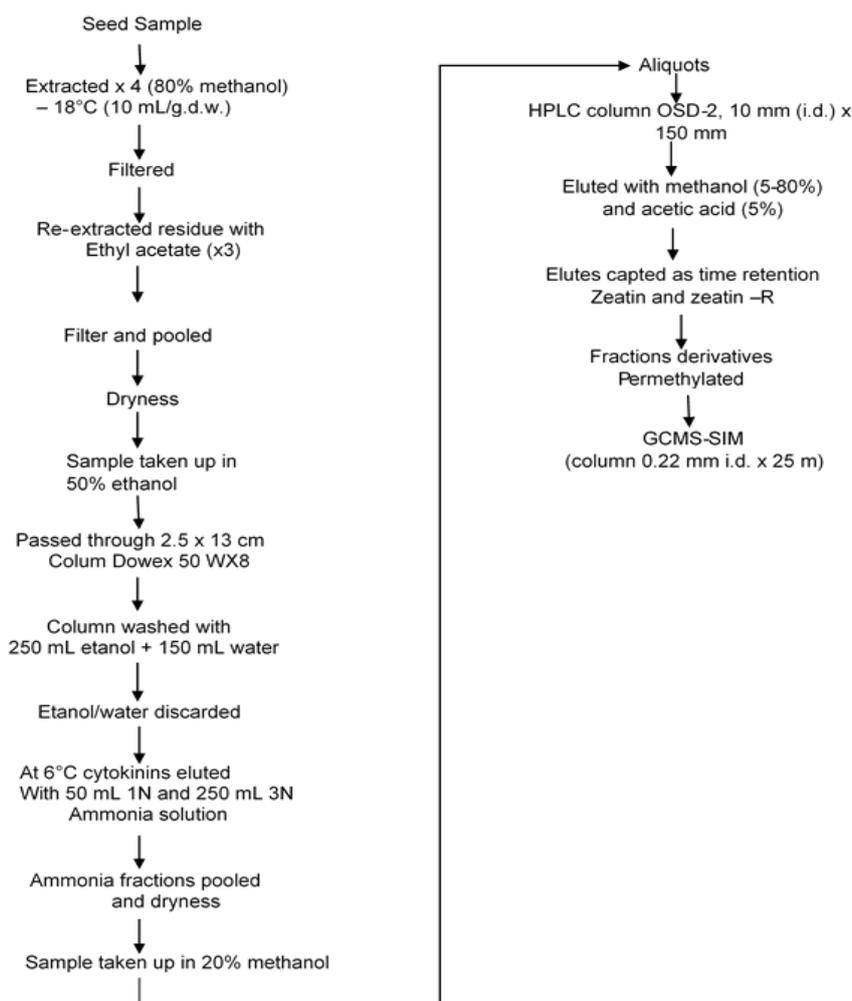


Fig. 2. Flow diagram outlining procedure used for the extraction and purification of seed extracts for cytokinins using HPLC and GCMS-SIM

3. RESULTS AND DISCUSSION

3.1 Endogenous Gibberellins

The analysis of mass spectrometry was focused on the prominent fragment ions in the peak corresponding with the retention time of authentic GAs as it has been described in great detail by Ramírez et al. [6]. Active and inactive gibberellins were both identified in seeds of saladette "SVTE 8832" tomato (Fig. 3).

The gibberellins extracted and eluted through a silicic column with ethyl acetate in n-hexane and identified by GCMS. The presence of biological inactive gibberellins A₉, A₁₂ and A₁₅ were found in the 10% ethyl acetate/n-hexane fraction. The biological active A₄ and A₇ were found in the 20% fraction; whilst, the inactive A₂₀ and A₅₃ in the 30% fraction; and the A₁₇ and A₄₄ in the 40%

fraction. A₁ which is highly active was detected in the 60% fraction (Fig. 4). Table 1 shows the frequency of gibberellins during the water seed imbibition time. The biological active gibberellins A₁, A₄, and A₇ were present at all times. The GA₉ appeared on days 2 and 3, GA₁₂ on day 1, GA₁₅ on day 2, GA₁₇ on day 3, GA₂₀ on days 2 and 3, GA₄₄ and GA₅₃ on day 3.

The identification in this study of gibberellins A₁, A₄, A₇, A₉, A₁₂, A₁₅, A₁₇, A₂₀, A₄₄ and A₅₃ may reflect the complicated role of these hormones in seed germination. The retention time intensity and ion number for each gibberellin show their particular chemical nature (Fig. 3). The extraction of each gibberellin through different percentage of solvents as seen in Fig. 4, reflect the polarity for particular hormone [7]. It is well established that exogenous application of GA₄ and GA₇ promote seed germination in tomato and several other crops [6,11]. GA₁ has also been reported

as seed germination promotor [7,12,13]. These three hormones move through different plant organs whereas the rest of GAs founded are classified as biological inactive [14,15,16]. On the basis of results obtained in the present work and previous data [17], it is possible to speculate as to which gibberellins are involved in the germination process. The rate of hydroxylation has been related to the movement of gibberellins [7]. Feeding labeled gibberellins to intact fruit has shown that some degree of hydroxylation is necessary for movement. GA₉ is immobile [18], but GA₃ [19,20] and GA₄ (Figs. 3, 4) both move from the fruit into spur tissue [6]. Therefore, it is likely that gibberellins A₉ and A₁₂ are immobilized due to their lack of hydroxylation. GA₄ moved out of fruits into the bourse-bud without being further hydroxylated [6]. On the basis of these results it seems that the more highly hydroxylated gibberellins such as GA₁, GA₄ and GA₇ [19,20] may be involved in the process of tomato seed germination; whereas the gibberellins A₉, A₁₂, A₁₅, A₁₇, A₂₀, A₄₄ and A₅₃ which as a result of lack of hydroxylation seem to be not so important for germination as those with more hydroxyl group (Fig. 4). Chen et al. [15], have proposed that tomato seed gibberellin 2-oxidases may play a direct role in germination and other physiological processes. Therefore, the presence of a range of gibberellins found in seed extracts opens further possibilities for explaining the way in which they exert their effect on the germination of tomato seeds.

3.2 Endogenous Cytokinins

The present study resulted in the identification of the endogenous cytokinins zeatin and zeatin-riboside (Figs. 5, 6). The presence of these two endogenous plant hormones (Fig. 7) was consistently evident at all times during the imbibition process (Table 2). The possible involvement of endogenous cytokinins in the process of seed germination is not well documented. Aremu et al. [21], have well documented the beneficial effects in yield and fruit quality when exogenous cytokinins are applied to several fruit crops. There is also evidence that cytokinins may be a key driver for seed yield [22], or act as a fruit growth promotor when deficiency of seed physiological function is present [23]. Matsuo et al. [24] established the role and regulation of cytokinins in tomato fruit development. Therefore, these researchers claim the importance of cytokinins in seed and fruit development. The findings of Z and ZR in this study are supported by the reports of Emery et al. [25] who found cis-zeatin, trans-zeatin and zeatin-riboside during seed development in white lupin and by Rijavec and Dermastia [26] who pointed out the importance of these cytokinins in developing seeds. Although the results on this study and the reports of other authors are useful [27,28], more research is need on the presence of cytokinins in tomato seeds and their possible role during seed germination.

Table 1. Gibberellins in seed tissue of saladette “SVTE8832” tomato. Gibberellins were identified by GC-MS

Water imbibition (days)	Gibberellins*									
	A ₁	A ₄	A ₇	A ₉	A ₁₂	A ₁₅	A ₁₇	A ₂₀	A ₄₄	A ₅₃
Saladette tomato “SVTE 8832”										
1	•	•	•		•					
2	•	•	•	•		•		•		
3	•	•	•	•			•	•	•	•

*Mean of three replicates

Table 2. Cytokinins in seed tissue of Saladette “SVTE 8832” tomato. Hormones were identified by GCMS-SIM

Water imbibition days	Cytokinins*	
	Zeatin	Zeatin-R
Saladette tomato “SVTE 8832”		
1	•	•
2	•	•
3	•	•

*Mean of three replicates

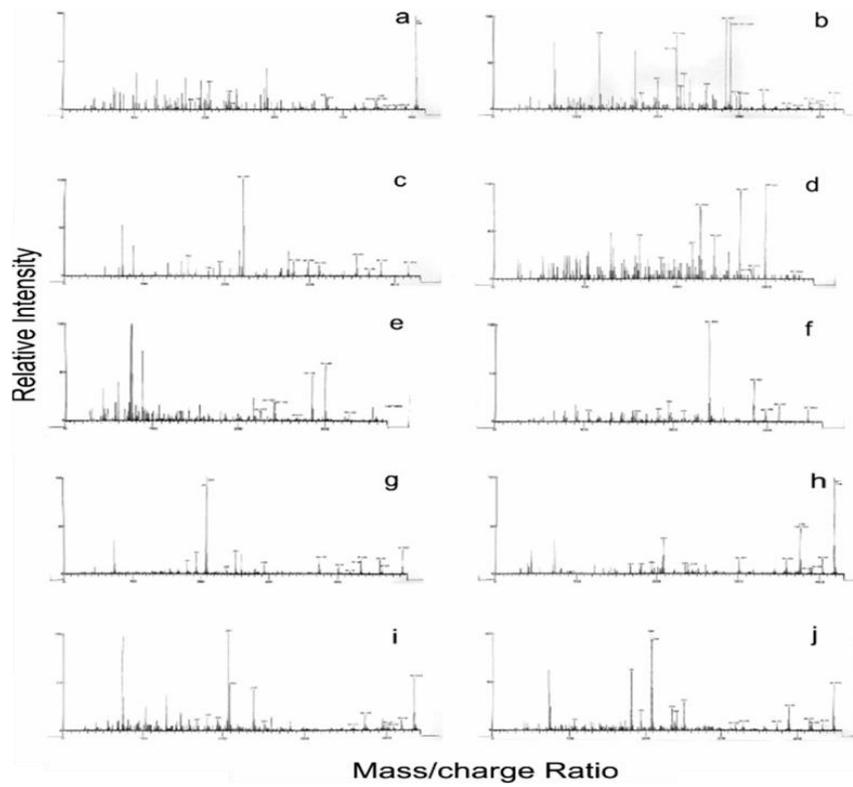


Fig. 3. Mass spectrum for GAs: A₁(a), A₄(b), A₇(c), A₉(d), A₁₂(e), A₁₅(f), A₁₇(g), A₂₀(h), A₄₄(i) and A₅₃(j) in saladette “SVTE8832” tomato seeds

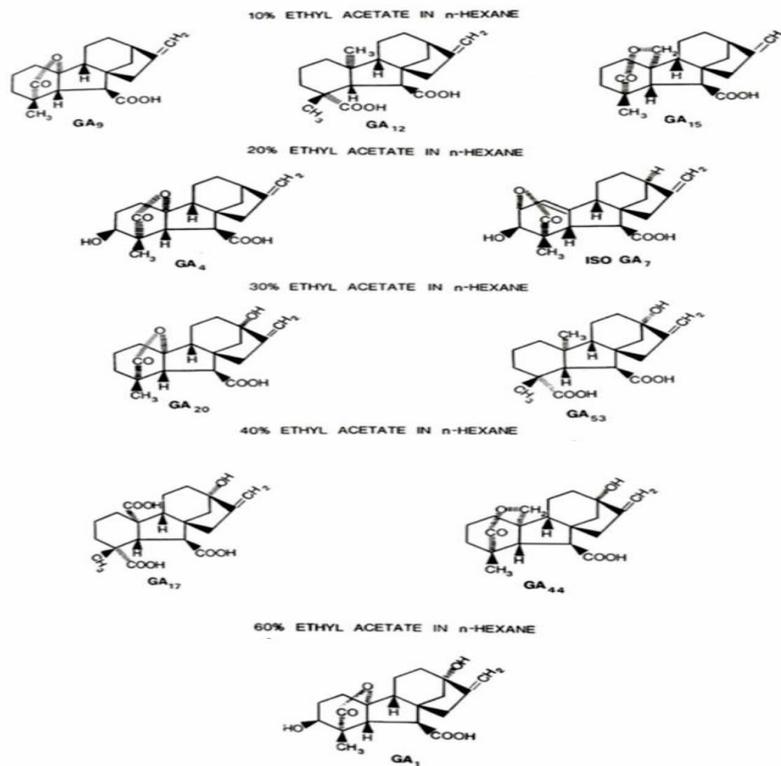


Fig. 4. Gibberellins in saladette “SVTE8832” tomato seeds. Silicic acid column eluted with ethyl acetate: n-hexane. Gibberellins were identified by GC-MS

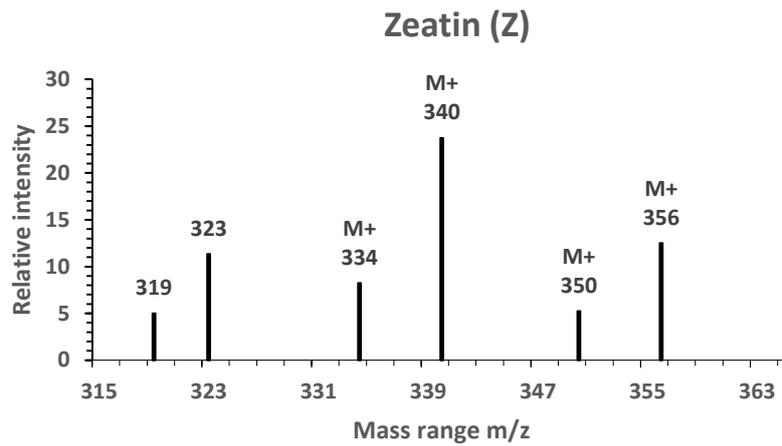


Fig. 5. Mass spectrum for zeatin (Z) in saladette “SVTE8832” tomato seeds

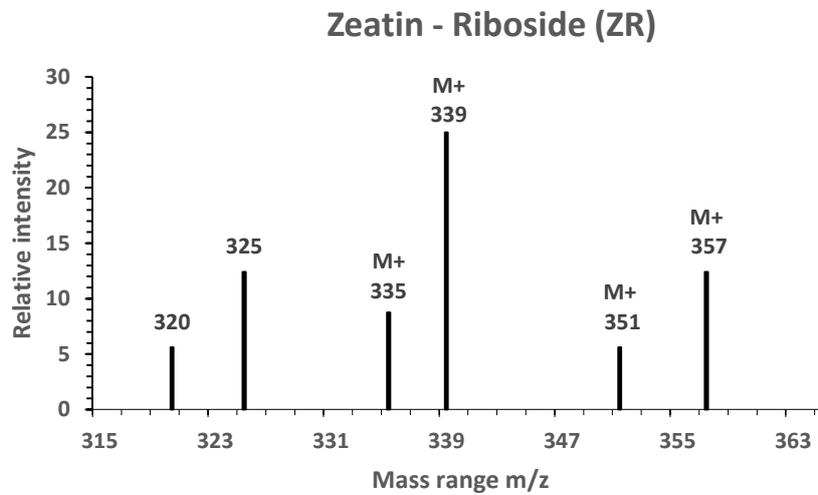


Fig. 6. Mass spectrum for zeatin-riboside (ZR) in saladette “SVTE8832” tomato seeds

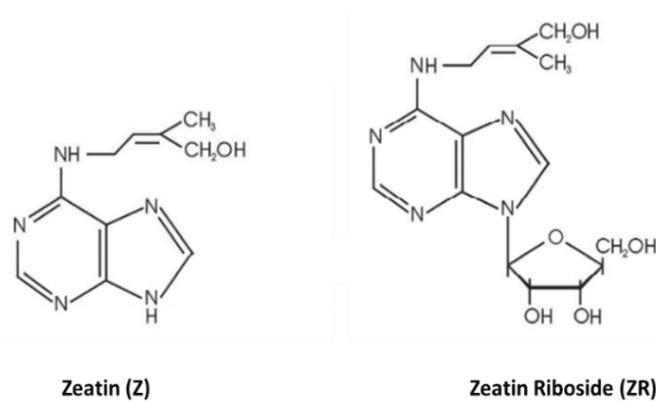


Fig. 7. Zeatin (Z) and zeatin-riboside (ZR) in saladette tomato seeds. Cytokinins were identified by GCMS-SIM

4. CONCLUSIONS

The endogenous gibberellins A₁, A₄, A₇, A₉, A₁₂, A₁₅, A₁₇, A₂₀, A₄₄, A₅₃ and the cytokinins zeatin and zeatin-riboside are present in saladette “SVTE8832” tomato seeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. SAGARPA. Planeación Agrícola Nacional 2017-2030. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. First ed. Ciudad de México: México: 20 pp. Spanish; 2020.
2. Ramírez H, Benavides A, Rangel EA. Identification of gibberellins in seeds of a Golden Delicious apple mutant. *Acta Horticulturae*. 2004;653:201-206.
3. Ramírez H. Identification of gibberellins in Golden Delicious apple seeds. *Acta Horticulturae*. 1993;329:95-97.
4. Ramírez H, Zavala-Ramírez MG, Sanchez-López A, Aguilar-Zarate P, Cristobal-Aguilar N, Rodríguez-García R, Jasso-Cantú D, Zermeño-Gonzalez A, Villarreal-Quintanilla JA, López-Fabián A. Tomato responses to biorregulators grown under greenhouse conditions. *International Journal of Plant & Soil Science*. 2016; 10(6):1-13.
5. Ramírez H, López-Fabian A, Peña-Cervantes E, Zavala-Ramírez MG, Zermeño-Gonzalez A. P-Ca, AG4/7 and 6-BAP en la fisiología y nutrición de tomate en invernadero. *Revista Mexicana de Ciencias Agrícolas*. 2018;9:747-759.
6. Ramírez H, Alvizo-Medrano BY, Melendres-Alvarez AI, Jasso-Cantú D, Villarreal-Quintanilla JA, Rodríguez-García R. Pomological characteristics and gibberellins identification on Golden Delicious Apple mutants. *Acta Horticulture*. 2018;1206:43-50.
7. Palni LMS, Summons RE, Letham DS. Mass spectrometric analysis of cytokinins in plant tissues. V. Identification of the cytokinin complex of *Datura innoxia* crown gall tissue. *Plant Physiology*. 1983;72:858-863.
8. Palni LMS, Tay SAB, MacLeod JK. GC-MS methods for cytokinins and metabolites. In HF Linskens, JF Jackson, eds. *Modern Methods of Plant Analysis*, Springer-Verlag. Heidelberg. 1986; 3:214-253.
9. Nandi SK, Palni LMS, Parker CAW. Dynamics of endogenous cytokinins during the growth cycle of a hormone-autotrophic genetic tumor line of tobacco. *Plant Physiology*. 1990;94:1084-1089.
10. Watanabe M, Bessho H. Seasonal changes of IAA and cytokinins of columnar type apple trees. *Acta Horticulturae*. 2008;774:75-80.
11. Bakrim A, Lamhamdi M, Sayah F, Chibi F. Effects of plant hormones and 20-hydroxyecdysone on tomato (*Lycopersicon esculentum*) seed germination and seedlings growth. *African Journal of Biotechnology*. 2007;6(2):2446. DOI: 10.5897/AJB2007.000-2446.
12. Alghonmeen O, Al-sharafa K, Allimoun M, Khleifat K, Al-Dein E, Al-ramamneh EA. Assessment of exogenous application of plant growth regulators on Crees seed germination and β -Galactosidase activity. *Plant Science Today*. 2020;7:743. DOI: 10.14719/pst.2020.7.2.743.
13. Singkaew J, Nishijima T, Photchanachai S. Effects of tomato seed maturity on seed quality and endogenous hormones. *Acta Horticulturae*. 2018;1208:355-362. DOI: 10.17660/Acta Hort.2018.1208.48.
14. Sirivasan A, Handa AK. Hormonal regulation of tomato fruit development: A molecular perspective. *Journal of Plant Growth Regulation*. 2005;24(2):67-82. DOI: 10.1007/s00344-005-0015-0.
15. Chen S, Wang X, Zhang L, Lin S, Liu D, Wang Q, Cai S, El-Tanbouly R, Gan L, Wu H, Li Y. Identification and characterization of tomato gibberellin 2-oxidases (GA2oxs) and effect of fruits specific SIGA2ox1 over expression on fruit and seed growth and development. *Horticulture Research*. 2016; 3:16059. DOI: 10.1038/hortres.2016.59.
16. Hoard GV. The role of seed derived hormones in the control of flowering in apple. *Acta Horticulturae*. 1978;80:93-103.
17. Ramírez H, Hoard GV. Effects of gibberellins on fruit bud initiation in apple. *Acta Horticulturae*. 1981;120:131-136.

18. Garcia-Martinez JL. Gibberellin metabolism and control of fruit growth. *Acta Horticulturae*. 1997;463:39-52.
19. Looney NE, Pharis RP. Gibberellins and reproductive development of tree fruits and grapes. *Acta Horticulturae*. 1986;179:59-71.
20. Pharis RP, King RW. Gibberellins and reproductive development in seed plants. *Annual Review Plant Physiology*. 1985;36:517-568.
21. Aremu AO, Fawole OA, Makunga NP, Masondo NA, Moyo NA, Buthelezi NMD, Amo SO, Spichal L, Dolezal K. Applications of cytokinins in horticultural fruit crops: Trends and future prospects. *Biomolecules*. 2020;10(9):1222. DOI: 10.3390/biom10091222.
22. Jameson PE, Song J. Cytokinin: A key driver of seed yield. *Journal of Experimental Botany*. 2016;67(3):593-606. DOI: 10.1093/jxb/erv461.Epub2015Nov1.
23. Ding J, Chen B, Xia X, Mao W, Shi K, Zhou Y, Yu, J. Cytokinin induced-parthenocarpic fruit development in tomato is partly dependent on enhanced gibberellins and auxin biosynthesis. *PLoS One*. 2013;8(7):e70080. DOI:10.1371/journal.pone.0070080.Print2013.
24. Matsuo S, Kikuchi K, Fukuda M, Honda I, Imanishi S. Roles and regulation of cytokinins in tomato fruit development. *Journal of Experimental Botany*. 2012; 63(15):5569-5579. DOI: 10.1093/jxb/ers207.Epub2012Aug3.
25. Emery RN, Qifu M, Atkins CA. The forms and sources of cytokinins in developing white Lupine seeds and fruits. *Plant Physiology*. 2000;123(4):1593-1604. DOI: 10.1104/pp.123.4.1593.
26. Rijavec T, Dermastia M. Cytokinins and their function in developing seeds. *Acta Chime Slovenia*. 2010;57:617-629.
27. Ramirez H, Sánchez-Canseco JC, Ramirez-Pérez LJ, Benavides A. Significance of hormones on flower bud initiation and fruit quality in Apple. Our expertise. *Acta Horticulturae*. 2014;1042: 73-77.
28. Steward FC, editor. *Plant Physiology 10: A Treatise: Growth and development*. 29th ed. Academic Press (NY): USA; 2012.

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