



Endogenous Hormones in Habanero Pepper Seeds

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Authors' contributions

This research was carried out in collaboration among all authors. Authors HR, UAMC, AIMA and MCCR 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: Habanero pepper (*Capsicum annuum* L.) cultivation is growing worldwide, mainly as a result of its high demand and nutritive contribution to human society. The presence of endogenous gibberellins and cytokinins in seeds of various vegetable crops has been related to a good germination; however, little is known on habanero pepper. The aim of this study was to search for the presence, identification and amount of gibberellins and cytokinins in seeds of habanero pepper cv "Jaguar".

Study design: Laboratory analysis for gibberellins and cytokinins were organized 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

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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 “Jaguar” habanero pepper 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 GC-MS and GCMS-SIM respectively.

Results: Gibberellins A₁, A₄, A₇, A₉, A₁₅, A₁₇ and A₄₄ were found in habanero seed tissue. The cytokinins zeatin and zeatin-riboside were also detected in analyzed habanero samples.

Conclusion: The endogenous gibberellins A₁, A₄, A₇, A₉, A₁₅, A₁₇, A₄₄, and the cytokinins zeatin and zeatin-riboside are present in habanero pepper cv “Jaguar” contributing to an improve seed viability, ensuring health and overall plant yield.

Keywords: Habanero pepper; seeds; gibberellins; cytokinins.

1. INTRODUCTION

Habanero pepper (*Capsicum annuum* L.) is a vegetable crop with an increasing demand in the national and international markets. The fruit of this vegetable is gaining importance as a result of its high nutrients, flavonoids and antioxidants content, in particular capsaicin [1,2]. In order to meet the high demand of this crop, it is necessary to apply new horticultural techniques which could contribute to increase both yield and fruit quality. On these basis, modern horticulture seeks technologies related to improve these referred components. Among them; seed germination treatment is a crucial approach towards meeting that goal. The use of exogenous hormones has proven to promote seed germination in habanero and other vegetable crops [3]. Gibberellic acid is frequently used in the range of 40-100 mgL⁻¹ to increase germination in jalapeño pepper [2] and in Golden Delicious apple seeds [4]. Prohexadione-ca at 50 mgL⁻¹ promotes seed germination and seedling growth in habanero pepper [4]. A healthy habanero pepper seedling derives from a high quality seed which often relies on germination capacity and on the important activities of endogenous hormones [2]. Gibberellins and cytokinins have been related to seed germination of various crops such as peppers, lupins and apple [4]. Hot pepper seed treatments with gibberellic acid and 6-benzyl amino purine in a range of 40-70 mgL⁻¹ increased 15 % in germination rate and seedling growth. The presence of endogenous gibberellins such as GA₄, GA₇ and GA₉ is reported in seeds of deciduous fruit crops such as apple and has been related to an improvement in germination and seedling development [5]. However, the presence of gibberellins and cytokinins in habanero pepper seeds is less documented

[6,7]. Therefore, the aim of this study was to determine the presence, identification and amount of endogenous gibberellins and cytokinins in seeds of habanero pepper cv “Jaguar”.

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

“Jaguar” habanero pepper seed samples (50 g dry weight, INIFAP, México, 2019 seed stock) 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 [8] is illustrated in Fig. 1. Purified habanero pepper 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. [9,10] and by Nandi et al. [11]. 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. [12]. Each technical sample was replicated three times using a complete randomized design and Tukey's test when applicable.

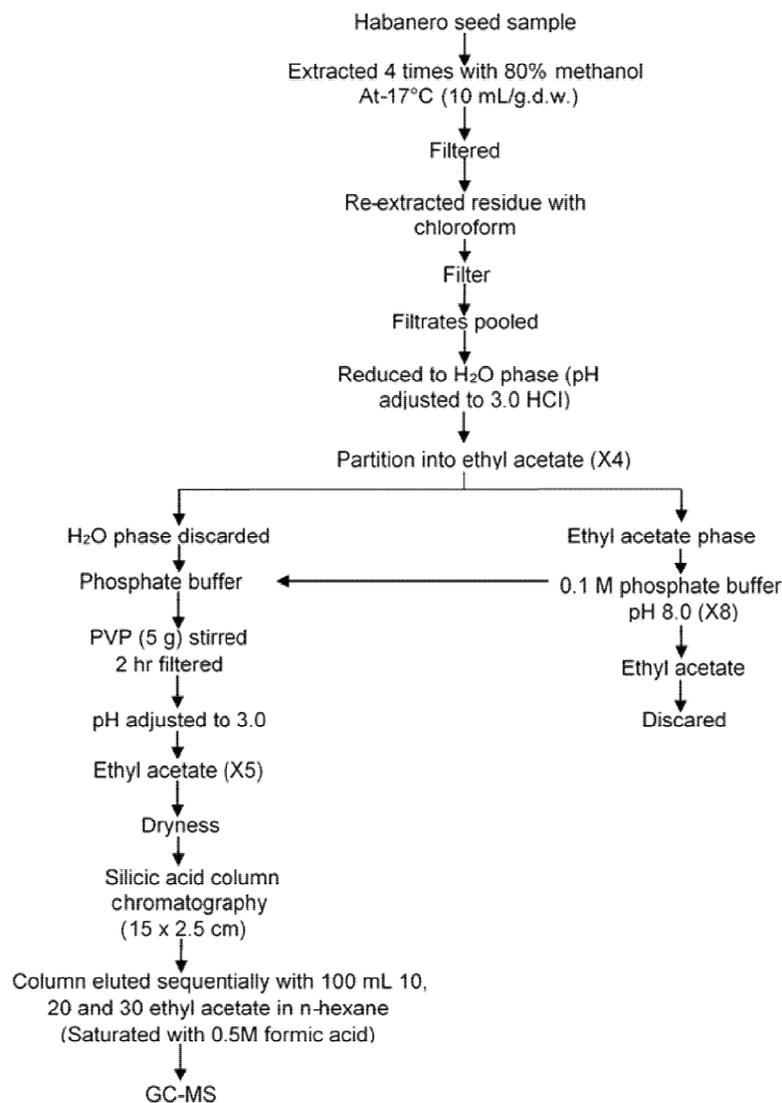


Fig. 1. Flow diagram outlining procedure used for the extraction and purification of habanero seeds extracts for gibberellin analysis using GC-MS

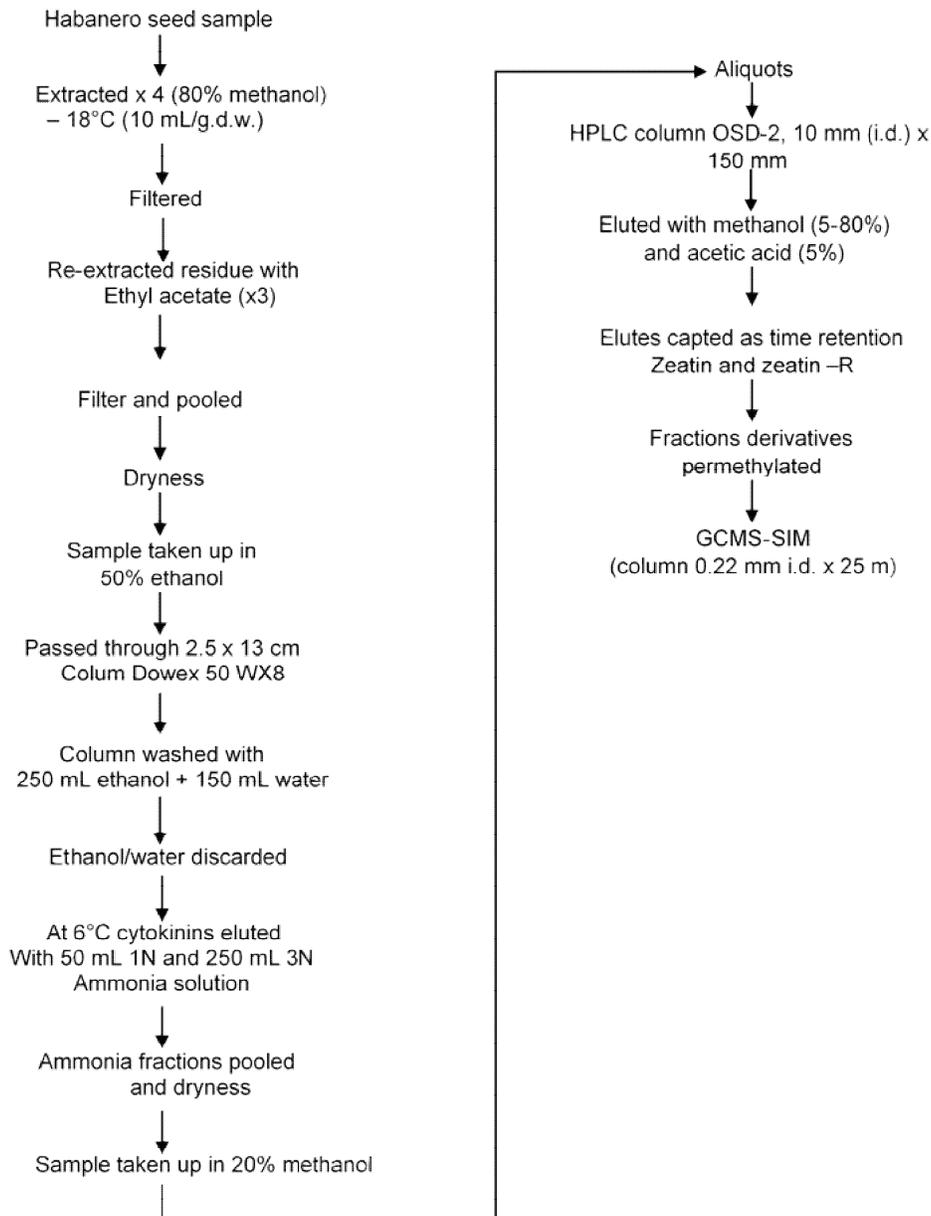


Fig. 2. Flow diagram outlining procedure used for the extraction and purification of habanero seeds extracts for cytokinins using HPLC and GCMS-SIM

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 gibberellins as it has been described by Ramirez et al. [8]. Table 1 shows the kovats retention index (KRI) for biologically active and inactive

gibberellins found in seed tissue of habanero pepper.

The gibberellins extracted and eluted through a silicic column with ethyl acetate in n-hexane and identified by GCMS are illustrated in Fig. 3. It can be seen the presence of biological inactive gibberellins A_9 and A_{15} in the 10% ethyl acetate/n-hexane fraction. The biological active A_4 and A_7 were found in the 20% fraction; whilst,

the inactive A₁₇ and A₄₄ in the 40% fraction. A₁ which is highly biological active was detected in the 60% fraction. Table 2 shows the presence 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 day 2, GA₁₅ on day 1, GA₁₇ on day 2 and GA₄₄ on day 3.

Table 3, shows the content of endogenous gibberellins in habanero seeds during the water imbibition phase. On day 1, GA₄ level was higher than GA₁ and GA₇; whereas, on days 2 and 3 the content of GA₁ was significantly less (Tukey $P \leq 0.05$) than GA₄ and GA₇. This difference was at least 4 ng. g⁻¹ fresh weight less when comparing with any of these two hormones.

Table 1. Retention index (kovats) for gibberellins in “Jaguar” habanero pepper seeds

Gibberellin	KRI ^a	Pattern of fragmentation and relative intensity (%)
GA ₁	2651	[506(100), 448(14), 377 (15), 375 (18)]
GA ₄	2488	[418 (21), 403 (2), 400 (12), 386 (25), 284 (100)]
GA ₇	2416	[416 (10), 193 (12), 179 (5), 155 (13)]
GA ₉	2295	[330 (5), 217 (37), 183 (19), 159 (45)]
GA ₁₅	2304	[344 (13), 211 (12), 195 (24), 183 (12), 159 (10)]
GA ₁₇	2417	[492 (26), 293 (81), 251 (26), 238 (5), 209 (100), 207 (90), 193 (11)]
GA ₄₄	2512	[432 (52), 251 (7), 238 (42), 207 (100), 193 (10), 180 (12)]

^aRetention index Kovats

Table 2. Gibberellins in seed tissue of habanero pepper cv “Jaguar”. Gibberellins were identified by GC-MS

Water imbibition (days)	Gibberellins*						
	A ₁	A ₄	A ₇	A ₉	A ₁₅	A ₁₇	A ₄₄
Habanero Pepper “Jaguar”							
1	•	•	•		•		
2	•	•	•	•		•	
3	•	•	•				•

*Mean of three replicates

Table 3. Endogenous gibberellins content in seed tissue of habanero cv “Jaguar”

Gibberellin	Imbibition days (ng. g ⁻¹ FW)			
	0	1	2	3
GA ₁	1a ^{z*}	4b	6b	9b
GA ₄	3a	8a	10a	13a
GA ₇	2a	5b	12a	17a

^zDifferent letters in same column indicate significant differences (Tukey $P \leq 0.05$); *Mean of three replicates

Table 4. Cytokinins in seed tissue of habanero pepper cv “Jaguar”. Hormones were identified by GC-MS-SIM

Water imbibition days	Cytokinins*	
	Zeatin	Zeatin-R
Habanero pepper “Jaguar”		
1	•	•
2	•	•
3	•	•

*Mean of three replicates

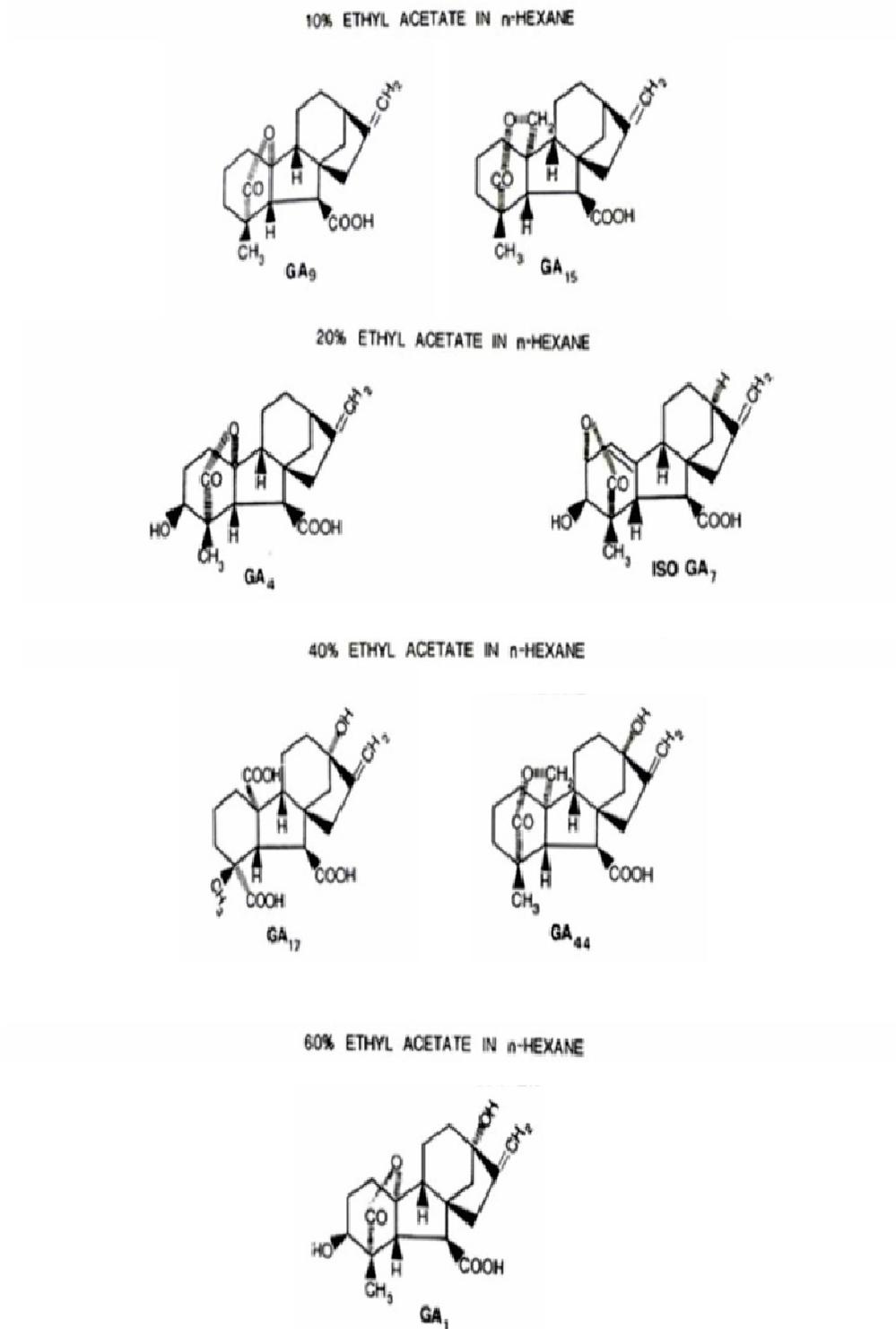


Fig. 3. Gibberellins in habanero pepper seeds. Silicic acid column eluted with ethyl acetate: n-hexane. Gibberellins appear as their degree of hydroxylation and polarity. Gibberellin structures as Ramirez et al model [8]

The identification of gibberellins A₁, A₄, A₇, A₉, A₁₅, A₁₇ and A₄₄ in habanero pepper seeds (Table 1, Fig 3) may contribute to enrich the possible role of these hormones in seed germination. The presence frequency (Table 2) and amount (Table 3) of gibberellins A₁, A₄ and A₇ during the seed imbibition time may reflect the importance of these hormones in the process of seed germination as has been proposed by several authors [3,13,14,15]. It is well established that when exogenous GA₄ and GA₇ are applied, both hormones promote seed germination in tomato, pepper and several other crops [3,6,16]. GA₁ has also been reported as seed germination promoter in various horticultural species [16,7]. These three hormones move throughout different plant organs whereas GAs A₉, A₁₅, A₁₇ and A₄₄ are classified as biological inactive hormones [3,8,13,14,15]. On the basis of results obtained in this research and previous data [14,17], it is possible to speculate as to which degree gibberellins may be involved in the germination process of habanero seeds. 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 an immobile hormone [8], but GA₃ [6,17] and GA₄ (Fig. 3) both move from the fruit into spur tissue [6]. Therefore, it is possible that gibberellins A₉, A₁₅, A₁₇, and A₄₄ are immobilized due to their lack of hydroxylation. It is known that [3 H]-GA₄ moved out of fruits into the bourse-bud without being further hydroxylated [8]. Thus, on the basis of these results it seems that the more highly hydroxylated, KRI (Table 1) and polarity (Fig. 3) gibberellins such as GA₁, GA₄ and GA₇ [10,17,18,19] may be involved in the process of habanero pepper seed germination; whereas the gibberellins A₉, A₁₅, A₁₇, and A₄₄ which as a result of lack of hydroxylation, seem to be not involved in the process of germination as those with more hydroxyl group (Fig. 3). Chen et al [20], have proposed that seed gibberellin 2-oxidases in some vegetable crops such as tomato may play a direct role in germination and other physiological processes. The frequency of presence during imbibition (Table 2) and the higher content (Table 3) of gibberellins A₁, A₄, A₇ may also reflect the physiological importance of these hormones in the process of seed germination. Therefore, the presence of a range of gibberellins found in seed extracts opens further possibilities for explaining the way in which they may exert their effect on the germination of habanero pepper seeds.

3.2 Endogenous Cytokinins

The present research in habanero pepper resulted in the identification of the endogenous cytokinins zeatin (Z) and zeatin-riboside (ZR). The mass spectrum for both molecules is illustrated in figures 4 and 5 respectively. The presence of these endogenous plant hormones (Fig. 5) was consistently evident at all times during the imbibition process (Table 4). The content of cytokinins varied during the imbibition seed period as shown in table 5 where the level of zeatin was significantly higher than zeatin-riboside on days 2 and 3. The possible involvement of endogenous cytokinins in the process of seed germination in habanero pepper is not well documented. The use of exogenous cytokinins provoked promotion in seed germination and fruit growth in jalapeño and habanero pepper [3,21,22]. Aremu et al. [23], reported the beneficial effects in yield and fruit quality when exogenous cytokinins are applied to several fruit crops. There is also some evidence that cytokinins could be a key driver force for seed yield [24], or may act as a fruit growth promotor when deficiency of seed physiological function is present [25]. Matsuo et al. [26] established the role and regulation of cytokinins in fruit development. These researchers supported 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. [27] who found cis-zeatin, trans-zeatin and zeatin-riboside during seed development in white Lupin and by Rijavec and Dermastia [28] 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 [29,30,31], more research is need on the presence of cytokinins in habanero pepper seeds and their possible role during seed germination.

Table 5. Endogenous cytokinins content in seed tissue of habanero cv “Jaguar”

Cytokinins	Imbibition days (ng. g ⁻¹ FW)			
	0	1	2	3
Zeatin	1a ^{z*}	5a	9a	12a
Zeatin-R	1a	3a	6b	10a

^zDifferent letters in same column indicate significant differences (Tukey P≤0.05); *Mean of three replicates

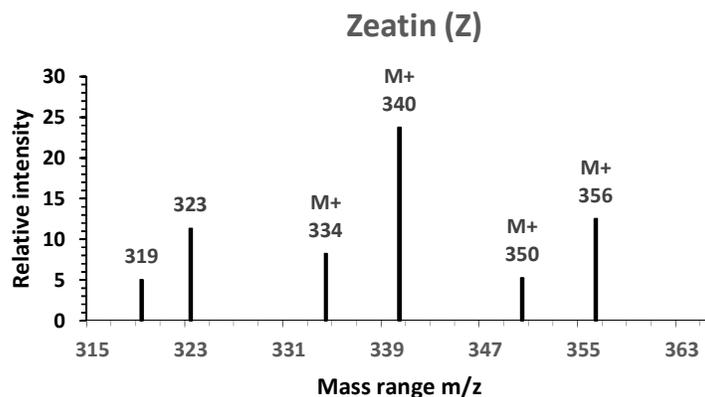


Fig. 4. Mass spectrum for zeatin (Z) in “Jaguar” habanero pepper seeds. Molecular ion region and fragment ions (M^+) are indicated

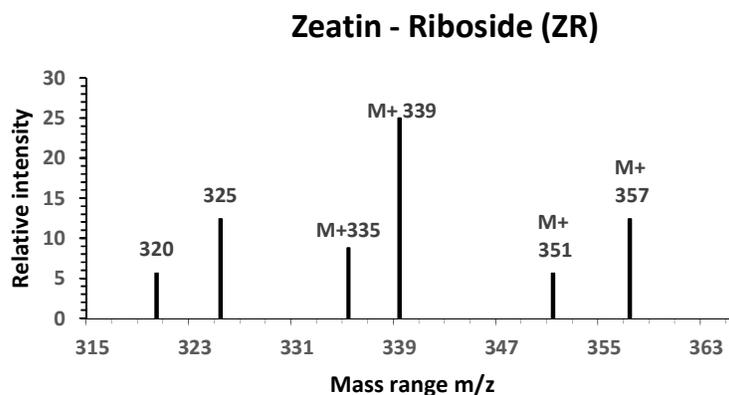


Fig. 5. Mass spectrum for zeatin-riboside (ZR) in “Jaguar” habanero pepper seeds. Molecular ion region and fragment ions (M^+) are indicated

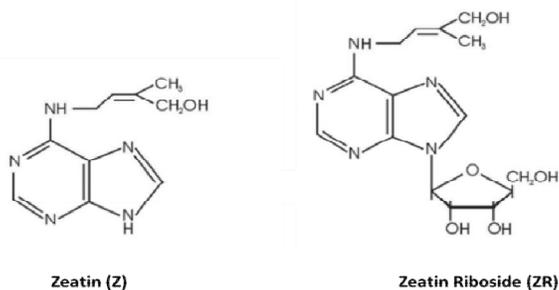


Fig. 6. Zeatin (Z) and zeatin-riboside (ZR) in “Jaguar” habanero pepper seeds

4. CONCLUSIONS

The endogenous gibberellins A_1 , A_4 , A_7 , A_9 , A_{15} , A_{17} and A_{44} were identified in “Jaguar” habanero

pepper seeds. Gibberellins A_1 , A_4 and A_7 are consistently present in seed tissue. GA_4 and GA_7 content remains higher during seed imbibition time.

The endogenous cytokinins zeatin and zeatin-riboside were identified in “Jaguar” habanero pepper seeds. Z and Z-R are consistently present in seed tissue and their content remains higher during seed imbibition time.

The results obtained in this study open further work towards which gibberellins and cytokinins are directly involved in habanero seed germination.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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