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Temperature and GA_3 as Modulating Factors in the Biosynthesis of Alkaloids during Imbibition and Early Development of *Annona* x *atemoya* Mabb. cv. 'Gefner' Seedlings

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Abstract: Alkaloids are products of the specialized metabolism of plants and temperature is a factor capable of modulating their biosynthesis. Species of the Annonaceae family biosynthesize alkaloids and present dormancy in their seeds, which can be overcome with the use of gibberellins. Therefore, the aim of this work was to evaluate whether temperature variations and the use of gibberellin in seeds affect the production of alkaloids during germination and early development of *Annona* x *atemoya* Mabb. cv. 'Gefner' seedlings. Results showed that the temperature of 30 °C associated with imbibition in water caused an increase in the production of total alkaloids and liriodenine and that the use of gibberellin decreased production. In addition, it was possible to identify the presence of nine other alkaloids with organ-specific distribution. The presence of none of them was induced by the effect of temperature or gibberellic acid. Therefore, it could be concluded that temperature variation and the use of GA_3 alter the biosynthesis of alkaloids, with high temperature causing increased concentration, but the use of GA_3 reducing production.

Keywords: benzylisoquinoline alkaloids; liriodenine; annonaceae; plant regulators; imbibition; gibberellic acid

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1. Introduction

Alkaloids are specialized metabolites containing nitrogen. The structural diversity of alkaloids is as wide as the range of their biological activities, such as antimicrobial, cytotoxic, antitumor, antiprotozoal, and antiviral actions [1–6]. Benzylisoquinoline alkaloids (BIAs) are a structurally diverse group of plant specialized metabolites. These alkaloids are typically isolated from plants of the order of Ranunculales, including Papaveraceae, Ranunculaceae, Berberidaceae, Menispermaceae, Magnoliaceae, and Annonaceae families [3].

The Annonaceae family has a great diversity of alkaloids, with reports of about 934 alkaloids, the most abundant being BIA-type alkaloids [7]. BIAs frequently found in annonaceous species are anonaine, asimilobine, isoboldine, isocoridine, liriodenine, stephalagine, nuciferine, atherospermidine, reticuline, laurotenine, lanuginosine, discretine, and xylopine [7–11]. Liriodenine alkaloid is perhaps the specialized metabolite most widely distributed in the Annonaceae, and this oxoaporphine is found in at least 86 genera and 240 Annonaceae species [3]. It is a molecule with biological activities, such as antitumor, antibacterial, antifungal, and antimalarial [3,7,12]. This BIA has been suggested as a defense molecule against phytopathogens in seedlings of the genus *Annona* [12,13].

The Atemoya (*Annona* x *atemoya* Mabb.) is an interspecific annonaceous hybrid between *Annona cherimola* Mill. and *Annona squamosa* L. that stands out due to the production and

commercialization of fruits. Its phytochemical investigation led to seven benzylisoquinoline alkaloids, including two aporphine (anonaine and asimilobine), three oxoaporphine (lanuginosine, liriodenine and lysicamine) and two proaporphine (pronuciferine and stepharine) [11,14].

Previous phytochemical studies with *Annona macroprophyllata* (≡*Annona diversifolia*), *Annona cacans*, and *Annona muricata* have shown that BIAs are present in different plant organs during the early seedling development [15–18], supporting the idea of a specific organ location and dependence on plant development [18]. Some of these alkaloids showed antimicrobial activity during early development [13]. The production of alkaloids with antimicrobial activities from the beginning of the germination process is a defense mechanism of the plant against biotic stress, being a strategy to guarantee its establishment in the environment and perpetuation of the species [15,19].

The alkaloids, biosynthesized from the specialized metabolism, represent a chemical interface between plants and the surrounding environment. Thus, their biosynthesis is often affected by environmental conditions, such as temperature, which can influence the composition and concentration of molecules [20–24]. However, the temperature affects not only the response of the specialized metabolism of plants, but also affects water absorption and the chemical reactions that regulate the metabolism involved in the germination process [25]. In addition, hormones such as gibberellins, which act in the development of the plant and in overcoming seed dormancy, can also affect the production of alkaloids [16].

Overcome dormancy occurs by altering the relationship between endogenous abscisic acid (ABA) and gibberellin (GA). The increase in GA (germination promoter) reduces the ABA (germination inhibitor) content, which results in increased degradation of the reserves, resulting in complete germination with primary root protrusion [26]. Several authors have reported the use of gibberellins (GAs) to overcome the dormancy of *Annona* seeds [26–29], as well as temperature variations altering the germination process [30–32]. However, the effect of temperature variation associated with the use of exogenous gibberellins in the production of alkaloids during the germination process and in the initial development of seedlings is a topic that deserves to be explored.

The evidence pointed out leads us to the objective of investigating whether temperature and the use of GA_3 modulate the synthesis of benzylisoquinoline alkaloids in *Annona* x *atemoya* Mabb. cv. 'Gefner' from seed imbibition to early seedling development.

2. Materials and Methods

2.1. Plant Material

Annona x *atemoya* Mabb. cv. 'Gefner' seeds were collected (May 2019) from ripe fruits in a commercial orchard in the municipality of Itapetininga, São Paulo, Brazil, and transported to the Plant Biology Sector, Biostatistics, Plant Biology, Parasitology and Zoology Department, São Paulo State University (UNESP), Botucatu, freezer at (4–5 °C) for 30 days, when the experiment was started.

2.2. Experimental Design and Experiment Implementation

The experimental design was completely randomized, in a 3 × 2 factorial scheme (temperatures × water and GA_3) with 4 replicates of 25 seeds per treatment. Treatments consisted of seeds imbibed in distilled water and gibberellic acid (GA_3) (ProGibb® 400, North Chicago, IL, USA) at a concentration of 500 mg L^{-1}, for 36 h [27] associated with different temperatures (20 °C, 30 °C, and 20–30 °C (alternating, 8 and 16 h, respectively) with 16 h light and 8 h dark photoperiod).

From a total of 4720 atemoya seeds, 100 were used (4 replicates of 25 seeds) before the imbibition (dry seeds), to do the alkaloid extraction (seed coat and endosperm). After the imbibed process for 36 h under different treatment conditions, the seeds (4620) were divided into 3 groups. In the first group (100 seeds/treatment = 600 seeds), seed coat and endosperm were submitted to the alkaloid extraction method. In the second group, (570 seeds/treatment = 3420 seeds) the seeds were kept in germinators under controlled conditions (previously described) until the formation of seedlings with 5 centimeters of

roots, separated from the formed structures (seed coat, endosperm, root, hypocotyl and cotyledons), which were subjected to the alkaloid extraction method. Seeds in the third group (100 seeds/treatment = 600 seeds) were submitted to the germination test.

2.3. Germination Test

For the germination test, 4 replicates of 25 seeds per treatment were used, with seeds imbibed in distilled water and GA_3 at different temperatures (20 °C, 30 °C, and 20–30 °C (alternating, 8 and 16 h, respectively)). After 36 h of imbibition, the seeds were placed in germination paper rolls (Germitest = germination test) moistened with 2.5 times the paper mass and kept in germinators under the conditions established in the treatments. The following variables were evaluated: germination percentage, speed, and average germination time.

2.4. Extraction and Quantification of Total Alkaloids and Liriodenine

Seed and seedling structures obtained by the different imbibition and germination conditions of each treatment were dried, ground, and weighed equally in four replicates per structure of each treatment. Samples were carbonated with saturated anhydrous sodium carbonate solution (Na_2CO_3) and allowed to dry completely in oven at 30 °C. Alkaloids were extracted with chloroform ($CHCl_3$) under stirring for 2 h and then filtered. The chloroformic phase was extracted with 1 M hydrochloric acid solution (HCl) and then alkalized with Na_2CO_3 until reaching pH 9.5. Once again, it was re-extracted with $CHCl_3$ and allowed to evaporate at room temperature in the dark until further analyses [15]. After complete $CHCl_3$ evaporation, samples were resuspended with the same solvent and evaluated in spectrophotometer with ultraviolet light source and the total alkaloid content was determined by spectrophotometry at 254 nm using liriodenine as standard in the preparation of the standard curve ($y = 0.0881x - 0.0112$, $R^2 = 0.9949$) [15].

After determining the total alkaloid content, the profile was evaluated and the quantification of liriodenine for each treatment was performed using ultra high performance liquid chromatography (UHPLC - Thermo Fisher-Scientific®, Waltham, MA, USA) and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software (Catalog number: CHROMELEON7, Waltham, MA, USA), with gradient pump and UV-Vis detector using C18 reverse phase column (150 × 4.6 mm and 5 µm particle diameter). The mobile phase was water (pH 3.5 with acetic acid) and methanol in a 30:70 isocratic model, with a flow rate of 1 mL/min, keeping the column temperature at 30 °C. Detection was performed in UV at 254 nm. For liriodenine quantification, calibration curves were performed by analyzing stock solution series ($y = 0.3595x - 0.0011$; $R^2 = 0.9989$ for samples with up to 10 µg of liriodenine in the extract and $y = 0.3658x + 1.142$; $R^2 = 0.9992$ for samples with more than 10 µg of liriodenine in the extract) [15]. The identification of asimilobine alkaloids, discretine, lanuginosine, laurotetanine, liriodenine, N-methyl-laurotetanine, norglaucine, oxoglaucine, reticuline, xylopine, and xylopinine was performed in comparison with the standards provided by Emmanoel Vilaça Costa and Jackson Roberto Guedes da Silva Almeida.

2.5. Statistical Analysis

The total alkaloids and liriodenine data were submitted to analysis of variance (Two-Way ANOVA) using the SigmaPlot software (Version 12.5, Chicago, IL, USA) and the means were compared by the Tukey test at 5% ($p < 0.05$) [33].

3. Results and Discussion

In general, it was observed that temperature was characterized as a factor of variation in alkaloid concentration (total alkaloids and liriodenine) in the early development stages (seed imbibition and early seedling). Furthermore, although the use of GA_3 in the imbibition solution has favored the overcoming of seed dormancy (which was expected), it reduced the biosynthesis of alkaloids during the early stage of seedling development.

In newly collected (dry) *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds, total alkaloids were detected in higher concentration in the endosperm (11.401 μg g^{-1}) than in the seed coat (2.057 μg g^{-1}), and liriodenine was found in endosperm (0.618 μg g^{-1}), and not found in the seed coat, which indicates that the storage of these molecules occurred before dispersion, during the seed formation process (seed organogenesis), thus differing from results published with species of the same genus, such as *Annona macroprophyllata* (≡*Annona diversifolia*), *A. purpurea*, *A. lutescens,* and *A. muricata*, where the biosynthesis of alkaloids begins at germination or when the seedling becomes photosynthetically active [3].

In the same way as total alkaloids, liriodenine was detected in the endosperm of newly collected *Annona* x *atemoya* seeds and imbibed seeds in GA_3 or H_2O for 36 h at different temperatures (20 °C, 30 °C and 20–30 °C) (Table 1). Studies carried out with *Annona cacans* [16] corroborate our results that liriodenine was found at a higher concentration in the endosperm, when compared to the seed coat.

Table 1. Concentration of total alkaloids (μg g^{-1} dry mass) and liriodenine (μg g^{-1} dry mass) in the structures (seed coat and endosperm) of *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds immersed for 36 h (solution with H_2O or GA_3) at different temperatures (20 °C, 30 °C and 20–30 °C).

		Total Alkaloid Concentration		Liriodenine Concentration	
	Temperature	**H_2O**	**GA_3**	**H_2O**	**GA_3**
Seed coat	20 °C	0.779 Aa *	1.771 Aa	ND	ND
	30 °C	1.553 Aa	1.346 Aa	ND	ND
	20–30 °C	1.481 Aa	1.833 Aa	ND	ND
Endosperm	20 °C	13.857 BCa	19.272 Aa	0.000 Ab	0.661 Aa
	30 °C	22.777 Aba	14.205 Ab	0.199 Aa	0.239 ABa
	20–30 °C	8.665 Ca	11.248 Aa	0.000 Aa	0.000 Ba

(*) Means followed by the same letter, uppercase in the column (temperatures in each imbibition solution), and lowercase in the row (imbibition solutions in each temperature), for each structure, do not differ from each other by Tukey's test at 5% probability. ND: not detected.

During the 36 h of imbibition, a period in which there is activation of the germinal metabolism [27,30,34], it was found that the temperature and the use of GA_3 altered the biosynthesis of alkaloids in seeds. Seeds kept in H_2O at 30 °C had the highest concentration of total alkaloids (22.777 μg g^{-1}), not differing from seeds treated with GA_3 and 20 °C, which was the treatment that promoted the highest liriodenine concentration in the endosperm during this period (Table 1).

In addition to the changes in alkaloid biosynthesis promoted by the treatments, it was possible to identify the presence of 10 alkaloids (asimilobine, discretine, lanuginosine, laurotetanine, liriodenine, N-methyl-laurotetanine, norglaucine, oxoglaucine, reticuline, xylopine, and xylopinine, in early seedling development (Tables 2 and 3).

Liriodenine was the only alkaloid found in all treatment conditions in seed and seedling structures of *Annona* x *atemoya* cv. 'Gefner', being also reported in studies with species of the same genus as *Annona cacans* (seedling), *Annona macroprophyllata* (≡*Annona diversifolia*) (seedling), *Annona crassiflora* (young plants), and *Annona emarginata* (adult) [8,9,12,16].

Alkaloids such as lanuginosine and N-methyl-laurotetanine were absent in the structures of seeds imbibed for 36 h, being identified from the seedling structures, regardless of temperature and imbibition condition. While the alkaloid xylopinine remained present only in the endosperm of the seeds and was absent in the seed coat, however, during the initial development of the seedling it was possible to observe its presence in all structures and treatments. The other alkaloids were present in at least one or more structures of seeds or seedlings, and the presence or absence associated with the temperature or imbibition condition was not evident.

Table 2. Presence (x) or absence ( ) of alkaloids in seedling structures (seed coat, endosperm, root, hypocotyl, and cotyledon) originating from *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds immersed for 36 h (with H_2O or GA_3) at temperatures of 20 °C, 30 °C, and 20–30 °C and kept at the same temperatures during early development.

Alkaloids	Seed Imbibition H_2O															Seed Imbibition GA_3													
	Seed Coat			Endosperm			Hypocotyl			Cotyledon			Root			Sead Coat			Endosperm			Hypocotyl			Cotyledon			Root		
	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30
Asimilobine							x	x	x							x	x	x			x		x				x		x	
Discretine																															
Lanuginosine		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Laurotetanine	x	x	x	x	x	x		x	x			x		x	x				x	x	x	x		x	x	x	x	x	x	x
Liriodenine	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
N-metil-Lauroteanine		x	x		x	x	x	x				x			x					x	x	x	x	x	x	x		x	x	x
Norglaucine		x																													
Oxoglaucine												x			x									x	x	x	x				
Reticuline			x																												
Xylopine	x	x	x		x	x	x	x				x		x	x			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Xylopinine	x	x	x	x	x	x			x	x	x	x	x		x		x		x	x	x	x	x	x	x		x	x	x		

Table 3. Presence (x) or absence ( ) of alkaloids in the seed structures (seed coat and endosperm) of Annona x atemoya Mabb. cv. 'Gefner' seeds immersed for 36 h (solution with H_2O or GA_3) at different temperatures (20 °C, 30 °C, and 20–30 °C) and dry seeds.

Alkaloids	Seed Dry		Seed Imbibition H_2O						Seed Imbibition GA_3					
	Endosperm	Seed Coat	Endosperm			Seed Coat			Endosperm			Seed Coat		
			20	30	20–30	20	30	20–30	20	30	20–30	20	30	20–30
Asimilobine	x	x		x			x	x	x	x		x	x	x
Discretine														
Lanuginosine	x								x					
Laurotetanine	x			x		x			x	x				
Liriodenine	x	x	x	x	x	x	x		x	x		x	x	x
N-metil-Lauroteanine														
Norglaucine														
Oxoglaucine				x					x	x				
Reticuline														
Xylopine				x										
Xylopinine	x		x		x				x	x	x			

The application of gibberellins is common in seeds for species of the Annonaceae family to overcome dormancy, as it alters the hormonal balance between Abscisic Acid (ABA) and Gibberellins (GAs) and promotes the biosynthesis of hydrolytic enzymes that degrade seed reserves, making energy available for embryo development and subsequent germination [26,28], which was observed in other studies with atemoya seeds [27,35] and this experiment, with significant dormancy-overcoming of seeds treated with GA_3. Temperature variation also affects, in general, the germination of Annona seeds, as alternating temperatures stimulate the highest germination percentage [25,30,31], including in atemoya seeds [32,35]. However, for atemoya seeds, it was observed in this experiment that temperature variation is no longer significant with the use of GA_3 (Table 4).

Table 4. Germinability (Germination percentage-% G; Percentage of dormant seeds-% D; Percentage of dead seeds-% M; Germination speed index-GSI; Mean germination time-MGT) of *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds immersed for 36 h (H_2O or GA_3) at temperatures of 20 °C, 30 °C, and 20–30 °C and kept at the same temperatures during germination.

	Temperature	**H_2O**	**GA_3**
% G	20 °C	15 Cb *	71 Aa
	30 °C	42 Bb	71 Aa
	20–30 °C	55 ABa	62 Aa
% D	20 °C	59 Aa	10 Ab
	30 °C	35 Ba	0 Ab
	20–30 °C	25 Ba	0 Ab
% M	20 °C	26 Aa	19 Aa
	30 °C	23 Aa	29 Aa
	20–30 °C	20 Aa	38 Aa
GSI	20 °C	0.209 Cb	0.805 Ca
	30 °C	1.063 Bb	2.155 ABa
	20–30 °C	1.618 ABa	1.676 Ba
MGT	20 °C	18.75 Ab	25.685 Aa
	30 °C	13.219 Ba	9.268 Bb
	20–30 °C	10.504 Ba	10.202 Ba

(*) Means followed by the same letter, uppercase in the column (temperatures in each imbibition solution), and lowercase in the row (imbibition solutions in each temperature), for each structure, do not differ from each other by Tukey's test at 5% probability.

The results obtained during imbibition reinforce the importance of GA_3 to overcome dormancy in *Annona* seeds (Table 4) and its use increased the concentration of alkaloids in the endosperm during imbibition (when associated with a temperature of 20 °C, similar to seeds imbibed in water at a temperature of 30 °C) (Table 1). However, it caused a reduction in the biosynthesis of alkaloids during seedling development (Table 5). These observations show that the stimulus provided by GA_3 directs the energy process to overcoming dormancy and promoting germination and not towards specialized metabolism, justifying the lower biosynthesis of alkaloids with the use of this regulator at different temperatures during imbibition.

When seeds were imbibed in H_2O, the germination process was slower (slower speed, longer average time, lower germination percentage and, therefore, higher dormancy) compared to seeds that received GA_3 (Table 4). However, in seeds imbibed in water, an increase in specialized metabolism was observed with the biosynthesis of alkaloids during imbibition and after germination, with an increase in the concentration of total alkaloids in all structures (seed coat, endosperm, root, hypocotyl, and cotyledons), with the only exception for temperatures of 20–30 °C (in the endosperm) and 20 °C (in the hypocotyl) (Table 5).

Table 5. Concentration of total alkaloids (μg g^{-1} dry mass) in seedling structures (seed coat, endosperm, root, hypocotyl, and cotyledon) originating from *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds immersed for 36 h (with H_2O or GA_3) at temperatures of 20 °C, 30 °C, and 20–30 °C and kept at the same temperatures during early development.

	Temperature	H_2O	GA_3
Seed coat	20 °C	27.291 Aa *	0.818 Ab
	30 °C	5.169 BCa	0.375 Aa
	20–30 °C	4.035 Ca	2.17 Aa
Endosperm	20 °C	168.218 Aa	13.609 Bb
	30 °C	46.133 BCa	28.24 Ba
	20–30 °C	34.151 Cb	83.5 Aa
Root	20 °C	147.039 Ca	44.782 Bb
	30 °C	342.344 Aa	119.647 Ab
	20–30 °C	173.474 BCa	88.002 ABb
Hypocotyl	20 °C	20.947 Cb	33.22 Aa
	30 °C	41.469 Ba	21.755 Ab
	20–30 °C	51.603 ABa	27.48 Ab
Cotyledon	20 °C	122.053 Aa	8.582 Bb
	30 °C	84.985 Aa	56.581 Bb
	20–30 °C	129.832 Aa	41.967 Bb

(*) Means followed by the same letter, uppercase in the column (temperatures in each imbibition solution), and lowercase in the row (imbibition solutions in each temperature), for each structure, do not differ from each other by Tukey's test at 5% probability.

In seedling structures, it was also possible to observe the presence of liriodenine, with the highest values found in the root and endosperm, 127.831 μg g^{-1} (H_2O, 30 °C) and 38.163 μg g^{-1} (GA_3, 20–30 °C), respectively, but also found in structures such as hypocotyl and cotyledons, which suggests mobility of these alkaloids or biosynthesis in different locations. In addition, liriodenine was detected in the coat of seeds that were still attached to the seedling (Table 6). Studies carried out in *Annona macroprophyllata* (≡*Annona diversifolia*) [15] did not detect the presence of liriodenine in the seed coat (kept attached to seedlings) and seedling cotyledons; being found only in root, hypocotyl, and endosperm structures.

Table 6. Liriodenine concentration (μg g^{-1} dry mass) in seedling structures (seed coat, endosperm, root, hypocotyl, and cotyledon) originated from *Annona* x *atemoya* Mabb. cv. 'Gefner' seeds immersed for 36 h (with H_2O or GA_3) at temperatures of 20 °C, 30 °C, and 20–30 °C and kept at the same temperatures during early development.

	Temperature	H_2O	GA_3
Seed coat	20 °C	0.000 Ba *	0.144 Ca
	30 °C	1.286 Aa	0.956 ABa
	20–30 °C	0.359 Ba	0.819 Ba
Endosperm	20 °C	6.921 Aa	2.112 Ba
	30 °C	18.656 Aa	13.116 Ba
	20–30 °C	7.011 Ab	38.163 Aa
Root	20 °C	27.453 Ba	13.210 Aa
	30 °C	127.831 Aa	26.912 Ab
	20–30 °C	46.304 Ba	22.658 Aa
Hypocotyl	20 °C	20.328 Aa	3.755 Bb
	30 °C	6.868 Aa	3.133 Ba
	20–30 °C	7.429 Aa	2.591 Ba
Cotyledon	20 °C	0.000 Bb	25.084 Aa
	30 °C	16.483 Ca	21.890 ABa
	20–30 °C	30.932 Aa	13.348 CBb

(*) Means followed by the same letter, uppercase in the column (temperatures in each imbibition solution), and lowercase in the row (imbibition solutions in each temperature), for each structure, do not differ from each other by Tukey's test at 5% probability.

This increase in alkaloid concentration during the early seedling development seems to be related to the defense capacity of plants in order to guarantee their establishment in the environment [13]. Thus, in addition to the different alkaloid concentrations obtained in seed structures at 36 h of imbibition, changes were also observed in the total alkaloid concentrations in seedling structures as a function of treatments (Table 5).

Among seedling structures, roots were those with the highest concentration of total alkaloids (342.344 μg g^{-1}) when seeds were imbibed in H_2O and kept at 30 °C, indicating that it is the structure with the highest biosynthesis of these molecules in atemoya seedlings during early development. Similarly, roots were also the structure with the highest alkaloid production in *Annona muricata* L. [18], *Annona lutescens* [17], and *Annona macroprophyllata* (≡*Annona diversifolia*) [15]. On the other hand, there was the effect of lower temperature (20 °C) on the increase of alkaloid concentrations in cotyledons, endosperm, and seed coat, still kept attached to developing seedlings.

The changes that occurred in alkaloid concentrations in different structures as a function of temperature, from the imbibition process to the early seedling development, provide information that contributes to a greater understanding of how this species adapts to environmental temperature variations. Although Christie, Alfenito, and Walbot [20] and Wallaart et al. [24] observed that low temperatures significantly influence the levels of specialized metabolites, which was also observed in this experiment in some seedling structures at 20 °C, the authors did not work with alkaloids.

4. Conclusions

Temperature and GA_3 cause alterations in the biosynthesis of total alkaloids and liriodenine from the beginning of the germination process to the early *Annona* x *atemoya* Mabb. cv. 'Gefner' seedling development. It could also be concluded that the use of the GA_3 regulator to break dormancy reduces the biosynthesis of alkaloids and liriodenine in the early development stages, while high temperature (30 °C) stimulates the biosynthesis of alkaloids in seedling roots.

Liriodenine, lanuginosine, laurotetanine, N-methyl-laurotetanine, xylopine, and xylopinine alkaloids have a more extensive presence in time and space; Liriodenine, lanuginosine, and laurotetanine are molecules that are formed during embryogenesis, while N-methyl-laurotetanine, xylopine, and xylopinine are formed during seed germination.

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