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# Gibberellic acid and warm incubation temperatures as germination stimulants in yellow kiwifruit seeds (*Actinidia chinensis* var. chinensis)

## Ácido giberélico y temperaturas cálidas de incubación como estimulantes de la germinación en semillas de kiwi amarillo (*Actinidia chinensis* var. chinensis)

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## ABSTRACT

The kiwifruit (*Actinidia chinensis* var. chinensis) is a deciduous fruit tree belonging to the *Actinidiaceae* family. Its industry in Mexico is still incipient, making it necessary to introduce new cultivars with diverse pulp colors and flavors. To achieve this, a population of seed-derived plants was used; however, these exhibited dormancy and low germination rates. This study aimed to evaluate pre-germination treatments to improve seed germination capacity. A completely randomized design with a factorial arrangement was applied, evaluating five concentrations of gibberellic acid ($GA_3$) [0, 1,000, 2,000, 4,000, and 6,000 ppm] in combination with three constant temperature levels (25, 30, and 35 °C) for 68 days. Prior to treatment application, seeds were stratified at 4 °C for 31 days and then immersed for 24 hours in their respective $GA_3$ solutions and water. Each experimental unit consisted of 50 seeds, with three replications. The variables evaluated included germination percentage (GP), mean germination rate (MGR), mean germination time (MGT), and days to dormancy release (DL), using ANOVA and Tukey's test ($P \leq 0.05$). Significant differences were found for all variables except MGT. The best results were obtained at 25 °C with any $GA_3$ concentration, where dormancy period was shortened (2.63 days), and both GP (6.35%) and MGR (0.5 seeds/day) increased. Constant temperatures above 30 °C negatively affected these variables.

**Keywords:**
Deciduous
Latency
Stimulation
Temperature

## RESUMEN

El kiwi (*Actinidia chinensis* var. chinensis) es un frutal caducifolio de la familia *Actinidiaceae* cuya industria en México aún es incipiente, por lo que se requiere la introducción de nuevos cultivares con pulpas de distintos colores y sabores. Para ello, se utilizó una población de plantas obtenidas por semilla, las cuales presentaron latencia y bajo porcentaje de germinación. El objetivo de este estudio fue evaluar tratamientos pre-germinativos para mejorar su capacidad germinativa. Se aplicó un diseño completamente al azar con un arreglo factorial, evaluando cinco concentraciones de ácido giberélico ($GA_3$) [0, 1.000, 2.000, 4.000 y 6.000 ppm], combinadas con tres niveles de temperatura constante (25, 30 y 35 °C) por 68 días. Previo a la aplicación de los tratamientos, las semillas fueron estratificadas a 4 °C durante 31 días y posteriormente sumergidas por 24 horas en las respectivas soluciones de $GA_3$ y agua. Cada unidad experimental estuvo compuesta por 50 semillas, con tres repeticiones. Se evaluaron las variables: porcentaje de germinación (PG), velocidad media de germinación (VMG), tiempo medio de germinación (TMG) y días a latencia (DL), utilizando ANOVA y prueba de Tukey ($P \leq 0,05$). Se encontraron diferencias significativas en todas las variables, excepto en TMG. Las mejores respuestas se obtuvieron a 25 °C con cualquier concentración de $GA_3$, donde se redujo el tiempo de latencia (2,63 días) y se incrementaron el PG (6,35 %) y la VMG (0,5 semillas/día). Temperaturas superiores a 30 °C afectaron negativamente estas variables.

**Palabras clave:**
Caducifolio
Latencia
Estimulación
Temperatura

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The kiwifruit (*Actinidia chinensis* var. chinensis), belonging to the *Actinidiaceae* family, is a deciduous climbing plant of the genus *Actinidia* (Windauer et al. 2016). In Mexico, the kiwifruit industry is still emerging, making it necessary to introduce new cultivars with different pulp colors and flavors to expand the domestic market supply (Maghdouri et al. 2021). Conventional or commercial kiwifruit propagation is typically carried out by cutting and/or grafting, using rootstocks obtained from seed-propagated plants. However, the presence of dormant embryos poses a challenge, as it increases seed population losses and complicates seedling uniformity (Maghdouri et al. 2021).

It is understood that seed dormancy is the inability of viable and intact seeds to complete germination, even under favorable environmental conditions (Gao and Ayele 2014). This physiological mechanism functions as an adaptive strategy in many plant species, allowing them to regulate the timing of germination to maximize seedling establishment success (Sekhukhune et al. 2018b). However, in agricultural contexts, dormancy represents a significant limitation for the uniform propagation of crops, which has prompted the evaluation of various techniques aimed at overcoming it (Escobar et al. 2015).

Among the most used methods is cold stratification, which involves exposing seeds to low temperatures (typically between 4 and 6 °C) for a set period. This treatment simulates winter conditions that many seeds require to activate their metabolic processes and break dormancy (Castillo et al. 2013; Sekhukhune et al. 2018b; Zhang et al. 2018).

Likewise, the use of plant growth regulators, such as gibberellic acids (GAs), has proven highly effective in overcoming dormancy. These phytohormones, belonging to the diterpenoid group, are involved in key processes of plant growth and development, including germination activation (Yamaguchi 2008).

Although more than a hundred gibberellins have been identified, only a few possess biological activity, with $GA_1$, $GA_3$, $GA_4$, and $GA_7$ being the most relevant. The exogenous application of $GA_3$ has been widely used, as it stimulates the expression of genes and enzymes associated with reserve mobilization, facilitating radicle emergence and seedling development (Yamaguchi 2008). In kiwifruit seeds, dormancy-breaking treatments have primarily involved the use of $GA_3$ at various concentrations, applied through 24-hour seed immersion prior to incubation (Sekhukhune et al. 2018a).

Bishwas et al. (2018) evaluated the germination response of three kiwifruit varieties (*Abbot*, *Allison*, and *Bruno*) pretreated with $GA_3$ concentrations of 2,000, 4,000, and 6,000 ppm. The results showed a significantly higher germination percentage in the *Bruno* variety (68.67%), followed by *Allison* (47.33%) at 6,000 ppm. *Bruno* also recorded the fastest average germination rate (0.059 seeds per day) with an average germination time of 17 days.

In another study, seeds of *A. arguta* and *A. chinensis* were stratified at 4 °C for 28 and 42 days, followed by treatments with $GA_3$ at concentrations of 500, 1,000, 1,500, 2,000, and 2,500 ppm (Sekhukhune et al. 2018b). Seeds stratified for 28 days showed germination percentages of only 8% in *A. arguta* and 20% in *A. chinensis.* However, the combination of cold stratification (28 days) followed by $GA_3$ treatments at 1,435 and 1,610 ppm resulted in optimal germination of 88% in *A. arguta*, with an average germination time of 23 days. In *A. chinensis*, optimal germination (80%) was achieved with $GA_3$ concentrations of 1,565 and 2,050 ppm, reducing the average germination time to 25 days (Kumar et al. 2020).

Based on this background, this study aimed to determine the most effective pre-germination treatment for stimulating germination in kiwifruit seeds (*Actinidia chinensis* var. chinensis) collected from Huatusco, Veracruz. Five different concentrations of gibberellic acid ($GA_3$) [0, 1,000, 2,000, 4,000, and 6,000 ppm], were evaluated in combination with three constant temperature levels (25, 30, and 35 °C) for 68 days. Prior to treatment application, all seeds were subjected to stratification at 4 °C for 31 days, followed by a 24-hour immersion in their respective $GA_3$ solutions and in water.

## MATERIALS AND METHODS

### Study area

The study was conducted in the seed laboratory of the Department of Phytotechnology at the Universidad Autónoma Chapingo, Mexico, located at 19°51' N latitude and 98°88' W longitude, at an altitude of 2,377 meters

above sea level (masl). The experimental activities were carried out during the winter period of 2022-2023 under controlled conditions that ensured the stability of environmental variables throughout the development of the research.

**Seed Acquisition**

Kiwifruit seeds (*Actinidia chinensis* var. chinensis) were used, obtained from ripe, yellow-fleshed fruits collected in the community of Elotepec, municipality of Huatusco, state of Veracruz, Mexico. This location is situated at an altitude of 1,851 masl, with geographical coordinates of 19°11'21" N latitude and 97°01'59" W longitude. The fruits were collected during the autumn of 2022, selecting those with an average weight of 70 to 90 g, free of physical damage and signs of disease, to ensure the quality of the seeds used in the study.

The seed extraction process consisted of cutting the fruits in half and manually extracting the seeds along with the attached pulp. Subsequently, this mixture was placed in plastic containers for fermentation at room temperature for three days, which facilitated the separation of the seeds from the pulp through the decomposition of surrounding tissues. After fermentation, the seeds were carefully washed with distilled water to remove any pulp residues and other impurities. Finally, they were dried in the shade on paper towels and stored in paper envelopes at room temperature until their use in the germination trials, following the methodology proposed by González-Puelles et al. (2018). To determine seed viability, the tetrazolium test was conducted using a representative sample of 20 seeds per replicate (three replicates). This analysis was carried out following the protocol described by Lastuvka et al. (2021) and Windauer et al. (2016).

**Experimental design**

The experiment was conducted using a completely randomized design with a factorial arrangement (5x3), where the effects of five levels of gibberellic acid concentration, combined with three incubation temperatures were evaluated. Prior to treatment application, all seeds were stratified at 4 °C for 31 days, followed by a 24-hour immersion in their respective $GA_3$ solutions and water. The methods and factors evaluated in the germination process are detailed in Table 1.

**Table 1.** Description of pre-germination treatments for kiwifruit (*Actinidia chinensis* var chinensis) seeds.

| Treatments | $GA_3$ [ppm] | Immersion time (h) | Incubation temperature (°C) |
|---|---|---|---|
| 1 | 0 (water) | 24 | 25±2 |
| 2 | | | 30±2 |
| 3 | | | 35±2 |
| 4 | 1,000 | 24 | 25±2 |
| 5 | | | 30±2 |
| 6 | | | 35±2 |
| 7 | 2,000 | 24 | 25±2 |
| 8 | | | 30±2 |
| 9 | | | 35±2 |
| 10 | 4,000 | 24 | 25±2 |
| 11 | | | 30±2 |
| 12 | | | 35±2 |
| 13 | 6,000 | 24 | 25±2 |
| 14 | | | 30±2 |
| 15 | | | 35±2 |

$GA_3$: Gibberellic acid.

Each experimental unit consisted of 50 seeds, uniformly distributed in Petri dishes (100 mm in diameter) with moistened filter paper. Each treatment was replicated three times simultaneously. Incubation conditions were kept constant using germination chambers with controlled temperature and 85% humidity, ensuring treatment homogeneity. The moisture of the filter paper was checked daily to prevent desiccation, and germination observations were recorded every two days over a period of 68 days after sowing.

**Stratification method**

Prior to the process with gibberellic acid and disinfection, all seeds were subjected to a stratification period in a cold chamber at 4 °C for 31 days. This treatment aimed to simulate winter conditions necessary to break seed dormancy, promoting the synchronization of metabolic processes associated with germination, as recommended by various studies on *Actinidia* species (Zhang et al. 2018).

**Disinfection process**

After stratification, the seeds were disinfected to prevent potential microbial contamination during the germination process. Initially, the seeds were immersed in 70% ethanol for 1 minute to eliminate surface contaminants. Subsequently, they were placed in a 1% sodium hypochlorite solution for 5 minutes, ensuring deep disinfection without compromising seed viability. Finally, four consecutive washes with sterile distilled water were performed to remove any residual disinfectant agents, following the methodology described by Zhang et al. (2018).

**Experiment management**

After stratification and disinfection, the seeds were treated with different concentrations of gibberellic acid ($GA_3$) [0, 1,000, 2,000, 4,000, and 6,000 ppm], according to the experimental treatments established by Çelik et al. (2006) and Kumar et al. (2020). Subsequently, the seeds were rinsed thoroughly with distilled water to remove any residual growth regulator.

The Petri dishes were placed in SEEDBURO® germination chambers under dark conditions for the first five days, at constant temperatures of 25±2, 30±2 and 35±2 °C, depending on the corresponding treatment. Throughout the experiment, the filter paper was kept consistently moist with daily applications of distilled water, ranging from 3 to 13 mL depending on hydration needs as seeds germinated and metabolic activity increased.

**Evaluated variables**

Seed germination was visually evaluated every two days over a period of 68 days after sowing, until germination stabilized, as no new germination events were recorded after day 57. The following variables were determined based on the collected data**:**

-Germination Percentage (GP) = Number of germinated seeds / total number of seeds, expressed as (%) (Maghdouri et al. 2021). According to Equation 1:

$$PG = \frac{ni}{N} \times 100\% \quad (1)$$

Where ni is the number of germinated seeds and N is the total number of seeds.

-Mean Germination Rate (MGR) = Expressed as germinated seeds per day (seeds per day) (Sharma et al. 2021).

-Mean Germination Time (MGT) = The time elapsed in days from when the seeds are sown until 50% of the germinated seeds are reached (Sharma et al. 2021). According to Equation 2:

$$MGT = \frac{\sum ni * di}{N} \quad (2)$$

Where ni is the number of seeds germinated on day ddd, di is the number of days since the start of the germination trial, and N is the total number of seeds germinated by the end of the trial.

**-Days to Latency (DL)**: The number of days elapsed between sowing and the start of germination of the first seed (Bishwas et al. 2018).

**Statistical analysis**

After completing the germination evaluations, the following response variables were calculated: germination percentage (GP), mean germination rate (MGR), mean germination time (MGT), and days to latency (DL). Since these variables did not follow a normal distribution according to the Shapiro-Wilk test ($P \leq 0.05$), a square root transformation was applied to normalize the data

and meet the assumptions required for the analysis of variance (ANOVA).

ANOVA was used to determine the existence of significant differences among the evaluated treatments, considering the factors of stratification, gibberellic acid concentration, incubation temperatures, and their interaction. Tukey's multiple comparison test at a 95% confidence level was applied to identify which treatments showed significant differences. All statistical analyses were performed using the Infostat software version 2020 (Di Rienzo et al. 2020).

## RESULTS AND DISCUSSION

According to the results obtained from the mean comparisons, significant differences ($P \leq 0.05$) were found in all evaluated variables, except for the mean germination time (MGT). This suggests that *Actinidia chinensis* var. chinensis seeds responded differently to the various constant temperature levels used during the incubation process (25, 30 and 35 °C).

It is noteworthy that at a temperature of 35 °C, germination was completely inhibited (0%), indicating that elevated temperatures induce thermal stress that prevents the activation of the metabolic processes necessary for germination (Fernández and Johnston 2006) (Table 2). These results are consistent with previous studies (Çelik et al. 2006), where temperatures above 30 °C showed negative effects on germination in other *Actinidia* species.

**Table 2.** Comparison of means for germination variables in kiwifruit (*Actinidia chinensis* var chinensis) seeds subjected to different temperatures.

| Temperature (°C) | GP | MGR | DL | MGT |
|---|---|---|---|---|
| 25 | 5.96[a] | 0.54[a] | 2.63[a] | 6.51[a] |
| 30 | 4.52[b] | 0.30[b] | 3.46[b] | 6.27[a] |
| HSD | 0.65 | 0.11 | 0.46 | 0.37 |

Different letters between treatments denote significant differences (Tukey test, $P \leq 0.05$); HSD: honestly significant difference; GP: germination percentage; MGR: mean germination rate; MGT: mean germination time; DL: days to latency.

The results obtained demonstrated that seeds incubated at 25 °C for 68 days achieved the highest values of germination percentage (GP) and mean germination rate (MGR), with 5.96% and 0.54 seeds germinated per day, respectively. Additionally, these seeds completed the germination process in the shortest time (DL=2.63 days), which represents a shorter duration than that reported by Çelik et al. (2006). These findings suggest that 25 °C provides optimal conditions for activating the physiological and biochemical processes involved in kiwifruit seed germination, promoting greater efficiency in reserve mobilization and enzymatic activity required for radicle emergence (Fernández and Johnston 2006; Rosabal et al. 2014).

When analyzing temperature as an independent factor, it was determined that once seed tissues are rehydrated at a constant temperature of 25 °C, they regulate the biochemical reactions necessary for germination more efficiently (Pérez 2003). This suggests that thermal stability within this range favors the synchronization of metabolic events prior to germination, preventing potential adverse effects associated with temperature fluctuations (Fernández and Johnston 2006). Similar behavior was observed by Çelik et al. (2006), indicating that as temperature increases, GP decreases. In particular, temperatures above 30 and 35 °C negatively affected these variables, possibly due to the denaturation of essential proteins for germination or an increase in the respiration rate that prematurely depletes the seed's energy reserves (Fernández and Johnston 2006).

On the other hand, various studies have demonstrated that exogenous application of gibberellic acid in pre-germination treatments has positive effects on kiwifruit seed germination (Çelik et al. 2006; Ahmad 2010; Zhang et al. 2018; Bishwas et al. 2018). This plant growth regulator plays a key role in activating hydrolytic enzymes, facilitating reserve degradation, and promoting cell elongation necessary for seedling emergence. However, although gibberellic acid is widely recognized as a stimulator of the germination process in this and other species, the results of this study did not show significant differences

in the evaluated variables across different concentrations of this phytohormone (Table 3). This suggests that the concentrations used in the experiment have the same capacity to effectively break kiwifruit seed dormancy and significantly increase the germination rate compared to the control, where values were null. It is possible that the doses evaluated have already reached a maximum response threshold, so additional increases in gibberellic acid concentration would not generate significant improvements in germination.

**Table 3.** Comparison of means for germination variables in kiwifruit (*Actinidia chinensis* var chinensis) seeds subjected to different concentrations of gibberellic acid ($GA_3$).

| Treatments $GA_3$ [ppm] | GP | MGR | DL (days) | MGT |
|---|---|---|---|---|
| 1,000 | 5.24[a] | 0.42[a] | 3.18[a] | 6.1[a] |
| 2,000 | 6.63[a] | 0.50[a] | 2.92[a] | 6.25[a] |
| 4,000 | 4.63[a] | 0.30[a] | 3.19[a] | 6.59[a] |
| 6,000 | 5.45[a] | 0.45[a] | 2.88[a] | 6.62[a] |
| HSD | 1.25 | 0.2 | 0.88 | 0.7 |

Different letters between treatments denote significant differences (Tukey test, $P \leq 0.05$); HSD: honestly significant difference; GP: germination percentage; MGR: mean germination rate; MGT: mean germination time; DL: days to latency.

Gibberellic acid produces a wide variety of responses in seed development and germination control, making it a key factor in inducing dormancy break after seed imbibition (Amador-Alférez et al. 2013). Its effect on seeds lies in reducing the environmental requirements necessary for germination, such as chilling hours, light conditions, and temperature, counteracting the effects of other plant hormones such as abscisic acid (ABA), and stimulating both germination and embryo growth (Gazzarrini and Tsai 2015).

Çelik et al. (2006) concluded that the influence of a single factor in germination studies is not sufficient. However, when it interacts or combines with other factors, the germination percentage (GP) increases. Therefore, in the double interaction of the factors evaluated in this study, significant differences were observed in all variables except for the mean germination time (MGT) (Table 4).

The treatments subjected to constant temperatures of 35 °C without the application of gibberellic acid were

**Table 4.** Comparison of means for germination variables in kiwifruit (*Actinidia chinensis* var chinensis) seeds subjected to the double interaction of the evaluated factors**.**

| Treatments | Description | GP | MGR | DL (days) | MGT |
|---|---|---|---|---|---|
| 4 | 1,000 ppm+25 °C | 6.13[ab] | 0.62[ab] | 2.87[a] | 6.21[a] |
| 5 | 1,000 ppm+30 °C | 4.34[b] | 0.29[ab] | 3.5[a] | 5.98[a] |
| 7 | 2,000 ppm+25 °C | 6.14[ab] | 0.58[ab] | 2.71[a] | 6.17[a] |
| 8 | 2,000 ppm+30 °C | 5.12[ab] | 0.41[ab] | 3.14[a] | 6.33[a] |
| 10 | 4,000 ppm+25 °C | 5.21[ab] | 0.38[ab] | 2.63[a] | 6.89[a] |
| 11 | 4,000 ppm+30 °C | 4.06[b] | 0.22[b] | 3.74[a] | 6.29[a] |
| 13 | 6,000 ppm+25 °C | 6.35[a] | 0.56[a] | 2.3[a] | 6.74[a] |
| 14 | 6,000 ppm+30 °C | 4.54[ab] | 0.27[ab] | 3.46[a] | 6.49[a] |
| | HSD | 2.15 | 0.38 | 1.51 | 1.2 |
| | CV | 14.5 | 16.44 | 17.58 | 6.7 |

Different letters between treatments denote significant differences (Tukey test, $P \leq 0.05$); HSD: honestly significant difference; CV: coefficient of variation.

not included in the tables due to the lack of satisfactory germination results (0%). Although previous studies, such as that of Çelik et al. (2006), reported germination rates of 45.3, 50, 51.7, and 48.2% under 35 °C conditions combined with 0, 2,000, 4,000, and 6,000 ppm of gibberellic acid, respectively, complete inhibition of germination was observed in the present study. This suggests that seeds of this *Actinidia chinensis* var. chinensis species do not require high temperatures to activate their metabolism and that thermal stress at 35 °C may have negatively affected seed viability.

In contrast, the interaction between a constant temperature of 25 °C and the different concentrations of gibberellic acid effectively broke dormancy and stimulated germination. In the absence of the growth regulator (control, data not shown), no germination was recorded (0%), while treatment 13 achieved a germination rate of 40.6%. These findings are consistent with those reported by Çelik et al. (2006), who determined that the optimal temperature for germination in other kiwifruit species, such as *A. arguta* and *A. kolomikta*, ranges between 20 and 25 °C.

Furthermore, Çelik et al. (2006) evaluated the interaction of three factors—temperature, growth medium, and gibberellic acid concentration—on the germination process of *Actinidia chinensis* cv. Hayward seeds. Their study analyzed four temperature levels (20, 25, 30, and 35 °C), three types of substrates (peat, perlite + heating humus, and soil mixture), and four gibberellic acid ($GA_3$) concentrations [1,000, 2,000, 4,000, and 6,000 ppm]. Results indicated that seeds treated with 6,000 ppm of $GA_3$ and sown in peat achieved the highest germination rate (79%), a value considerably higher than that obtained in the present study (40.6%).

Discrepancies between the two studies could be attributed to differences in seed genetics, experimental conditions, or the interaction between uncontrolled environmental factors. These results highlight the importance of evaluating multiple factors simultaneously to optimize kiwifruit seed germination, emphasizing the crucial role of temperature and gibberellic acid in the germination process.

## CONCLUSION

The interaction between the application of gibberellic acid ($GA_3$) and a constant incubation temperature of 25 °C significantly promoted the germination process of kiwifruit seeds (*Actinidia chinensis* var. chinensis) collected in Huatusco, Veracruz. This pre-germination treatment proved to be the most effective, as it reduced the dormancy period and increased both the germination percentage and the mean germination rate, without notable differences among the $GA_3$ concentrations evaluated. In contrast, temperatures above 30 °C had a negative effect on all germination variables, completely inhibiting germination. These findings reaffirm the effectiveness of combining $GA_3$ with controlled thermal conditions as a viable strategy to overcome seed dormancy in kiwifruit under tropical environments. Further research is recommended on incubation conditions below 25 °C and intermediate $GA_3$ concentrations (2,000 and 4,000 ppm) to refine propagation protocols and support the establishment of cultivars adapted to Mexico's agroclimatic conditions.

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## CONFLICT OF INTERESTS

The author declares no conflict of interest.

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