



## *In vitro* development of *Cattleya* spp. under different vitamin concentrations

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**ABSTRACT:** Vitamins belong to a group of organic nutrients essential in small quantities to life, performing several functions in metabolism and as antioxidants or resistance inducers. The lack of vitamins can lead to numerous developmental and metabolic problems, and excess may also be toxic. Despite their relevance, studies on the role of vitamins in the *in vitro* development of orchids remain scarce, especially for the genus *Cattleya*, which includes species of high ornamental and conservation value. This study aimed to evaluate the influence of B vitamins, Thiamine (B<sub>1</sub>), Nicotinamide (B<sub>3</sub>), and Pyridoxine (B<sub>6</sub>) on the development and seedling growth of *Cattleya labiata*, *Cattleya walkeriana*, and *Cattleya brevicaulis* during 120 days. The seeds were obtained from Universidade do Oeste Paulista (UNOESTE) Orchid Seedbank. In the Tissue Culture Lab at UNOESTE, the experiment was carried out in an *in vitro* half-strength MS medium for seedlings, and the following vitamins were tested: 0.025, 0.05, 0.1, and 0.2 mg L<sup>-1</sup> for Thiamine, and 0.125, 0.25, 0.5, and 1 mg L<sup>-1</sup> for Pyridoxine and Nicotinamide. The increment in fresh mass at 30-day intervals, dry mass at the end, shoot and root length, and the number of shoots and roots were evaluated. The complex B vitamins, Thiamine, Nicotinamide and Pyridoxine exhibited isolated effects in orchid seedling growth. It is recommended to reduce the Thiamine concentration to 0.025 mg L<sup>-1</sup>, decreased the Pyridoxine concentration to 0.125 mg L<sup>-1</sup>, and do not add Nicotinamide to the medium.

**Key words:** nicotinic acid, orchid, pyridoxine, thiamine, micropropagation.

## Desenvolvimento *in vitro* de *Cattleya* spp. em diferentes concentrações de vitaminas

**RESUMO:** As vitaminas pertencem a um grupo de nutrientes orgânicos, sendo essenciais em pequenas quantidades a qualquer ser vivo, desempenhando funções diversas no metabolismo e atuando como antioxidantes e indutores de resistência. A carência das vitaminas pode acarretar diversos problemas de desenvolvimento e metabolismo, assim como o excesso pode ser tóxico. Apesar de sua relevância, estudos sobre o papel das vitaminas no desenvolvimento *in vitro* de orquídeas permanecem escassos, especialmente para o gênero *Cattleya*, que inclui espécies de alto valor ornamental e de conservação. Este estudo teve como objetivo avaliar a influência das vitaminas do complexo B, tiamina (B<sub>1</sub>), nicotinamida (B<sub>3</sub>) e piridoxina (B<sub>6</sub>) no desenvolvimento e crescimento de plântulas de *Cattleya labiata*, *Cattleya walkeriana* e *Cattleya brevicaulis* durante 120 dias. As sementes foram obtidas do Banco de Sementes de Orquídeas da Universidade do Oeste Paulista (UNOESTE) e o experimento foi realizado no Laboratório de Cultura de Tecidos da UNOESTE, com meio de cultura MS à meia concentração, sendo as seguintes vitaminas testadas: 0,025, 0,05, 0,1 e 0,2 mg L<sup>-1</sup>, e 0,125, 0,25, 0,5 e 1 mg L<sup>-1</sup> para piridoxina e nicotinamida. Foram avaliados parâmetros de crescimento por meio de massa fresca a cada 30 dias, que são: massa seca, comprimento da parte aérea e da raiz e número de brotos para cada uma das espécies. Os resultados mostram que as vitaminas do complexo B, Tiamina, Nicotinamida e Piridoxina apresentaram efeito isoladamente no crescimento das mudas de orquídeas. Recomenda-se ajustar a concentração de Tiamina para 0,025 mg L<sup>-1</sup>, de Piridoxina para 0,125 mg L<sup>-1</sup> e não acrescentar Nicotinamida ao meio.

**Palavras-chave:** ácido nicotínico, orquídeas, piridoxina, tiamina, micropropagação.

## INTRODUCTION

Orchids were cultivated for a long time for beauty or medicinal purposes. However, the overcollection for these uses or habitat destruction for anthropogenic action has driven many species and their populations to extinction or a highly vulnerable position (FAY, 2018). In nature, orchid seeds are unable to germinate alone, and they need an association with a specific fungus to germinate that

receives organic molecule nutrients and provides minerals and other molecules to the seedlings (RASMUSSEN et al., 2015; YAM & ARDITTI, 2009). As a consequence, many species have a low reproductive success, despite the high number of produced seeds, being necessary either for plants reintroduction or commerce, the mass propagation of *in vitro* plants via symbiotic with the aim of a fungus, or asymbiotic germination, in artificial media trying to mimic the nutrients supplied by the

fungus and to favour the species (MAHENDRAN et al., 2013; RASMUSSEN et al., 2015). Thereby, complex organics such as malt extract, coconut milk, and banana pulp are added to the culture medium, which also contains vitamins (thiamine, pyridoxine and nicotinic acid), sugars and hormones (ABRAHAMIAN & KANTHARAJAH, 2011; GNASEKARAN et al., 2010).

Vitamins are substances needed in small amounts, which are employed as cofactors in enzymes and as antioxidants, enhancing the enzyme activities or protecting the organisms against ROS damage (ABRAHAMIAN & KANTHARAJAH, 2011). Under normal conditions, plants can produce the amount needed of vitamins or, in the case of orchid seeds, receive it from the symbiont (HIJNER & ARDITTI, 1973; RASMUSSEN et al., 2015). Conversely, when submitted to *in vitro* cultivation, as the explant does not have large amounts of reserves, there is a need to add them to complement the requirements for plant growth and development in the culture medium (ABBASZADEH et al., 2018; ABRAHAMIAN & KANTHARAJAH, 2011).

The media formulations are variable, which will impact the growth rate for orchids and can be species-specific while being influenced by characteristics such as geographical differences (tropical vs. temperate) and life traits (epiphytic vs. terrestrial) (DIANTINA et al., 2020); we must consider this when choosing a formulation as these plants may take from four to six years to first flower and to be commercialized. Even though living organisms can produce their vitamins, sometimes this amount may not be adequate, requiring external supplementation.

Thiamine (B1), Nicotinic acid (B3) and Pyridoxine (B6) are essential for many metabolic reactions (ABRAHAMIAN & KANTHARAJAH, 2011; VANDERSCHUREN et al., 2013) and are involved in the tricarboxylic acid cycle, amino acid biosynthesis, hormone degradation and stress tolerance (GOYER, 2010; MIRET & MUNNÉ-BOSCH, 2014). These vitamins are present in the composition of synthetic culture media for plant growth; however, there is no consensus on which vitamins are essential. Although vitamins play crucial roles in plant metabolism, there is a significant lack of specific studies on the genus *Cattleya*. The existing literature on the influence of vitamins on the *in vitro* development of *Cattleya* is limited, highlighting the need for further research in this area. Studies suggested that the addition of vitamins to the culture medium can be beneficial for root development

(VILLA et al., 2014), whereas vitamin deficiency can lead to reduced growth rates and lower biomass production (RODRIGUES et al., 2012). The lack of detailed data on the optimal concentrations and specific effects of these vitamins on the metabolism of *Cattleya* underscores the importance of additional investigations to optimise the *in vitro* cultivation of these species.

This project evaluated the effect of the vitamins Thiamine, Nicotinamide and Pyridoxine in the development and growth of *Cattleya labiata*, *Cattleya walkeriana*, and *Cattleya brevicaulis* during 120 days.

## MATERIALS AND METHODS

Seedlings of *Cattleya labiata* Lindl., *Cattleya walkeriana* Gardner and *Cattleya brevicaulis* (H.G. Jones) van den Berg were used. Seeds were obtained by cross-pollination between different plants and stored in the Universidade do Oeste Paulista (UNOESTE) Orchid Seed Bank. All used species are endemic to Brazil, with *Cattleya labiata* and *Cattleya walkeriana* being considered vulnerable (FLORA DO BRASIL, 2020).

### Germination

Germination was performed as described by MACHADO NETO & CUSTÓDIO (2005), except that they were sown in on half-strength nutrient concentration MS medium (MURASHIGE & SKOOG, 1962) containing 30 g L<sup>-1</sup> sucrose and 10 g L<sup>-1</sup> agar. The pH was adjusted to 5.6 before autoclaving at 1 atm, 121 °C for 20 minutes. The seeds were considered germinated when the embryo expanded through the testae and turned green, i.e., stage 1 (SEATON & RAMSAY, 2005). The germinated seeds were counted weekly until germination was stable. The seeds were evaluated in three marked fields in each Petri dish. Images were captured with a Sony DSC-P10 digital camera manually coupled to a stereomicroscope eyepiece lens.

### Vitamins assessment for growth

After germination and initial growth, the seedlings were individualized and transferred to the same media as used during germination. Vitamins were evaluated isolate in different concentrations: 0.025; 0.05; 0.1 and 0.2 mg L<sup>-1</sup> for Thiamine, and 0.125; 0.25; 0.5 and 1 mg L<sup>-1</sup> for Pyridoxine and Nicotinamide. The myo-inositol and glycine concentrations were maintained in all treatments. Seven repetitions with 5 seedlings were used in each

treatment for 120 days. Positive and negative controls were also used. The negative control was used with all salts except thiamine, pyridoxine, and nicotinamide. For the positive control, half macronutrients MS was used, all vitamins at half concentration (Table 1).

*Cattleya labiata* and *C. walkeriana* seedlings were individualized three months after germination, whereas *C. brevicaulis* seedlings were five months after germination. Fresh mass, length of the largest root, shoot length, number of shoots, and number of roots were evaluated monthly. At the beginning and end of the experiment, the dry mass of all treatments was assessed.

For the evaluation of fresh mass, a precision electronic scale chemically disinfected inside a laminar flow chamber was used; the initial and final shoot and root lengths were measured using a calliper graduated in millimetres; the number of shoots and the number of initial and final roots were manually verified. Every 30 days, seedlings were evaluated and transferred to flasks containing new culture medium. After the last weighing, the plants were dried in fine paper bags for 24 h in a micro processed drying oven, with a controlled temperature of 65 °C and forced air circulation, to subsequently measure the dry mass of each seedling per flask on a precision electronic balance. The results were compared with a sample used to determine the initial dry mass.

The experiment was designed as completely randomized. All data were subjected to ANOVA using the F test and means compared using the Tukey test at 5% probability. When applicable, polynomial regression of the data was performed (FERREIRA, 2011).

## RESULTS

All vitamins showed cumulative and increasing effects over the time studied, which can be observed by the regressions, which were all significant (Table 2). The behaviour of *Cattleya*

*labiata* seedlings in the different treatments can be seen in table 2. There was no difference between the Positive control, Negative control, and Thiamine concentrations in the growth of *Cattleya labiata* seedlings. For Pyridoxine, there was a concentration effect observed at 90 and 120 days (Table 2). The commonly used concentration (0.25 mg L<sup>-1</sup>), present both in the Positive control and the treatment with the isolated vitamin, did not statistically differ from half the concentration (0.125 mg L<sup>-1</sup>) at 120 days. The highest concentrations (0.5 and 1 mg L<sup>-1</sup>), as well as the Negative control, performed worse than the others. For Nicotinamide at half concentration (0.125 mg L<sup>-1</sup>) and full concentration (0.25 mg L<sup>-1</sup>), as well as the positive control, the results were statistically equal. The highest levels showed an inhibitory effect with a statistically equal result.

For *Cattleya labiata*, in the variables length of the largest root, number of roots, number of sprouts and dry mass, there was no statistical difference between the treatments with the different levels of Thiamine. However, in shoot length, the Negative control was numerically inferior to the other treatments of this vitamin, although there was no statistical difference between this one, the concentrations 0.025, 0.1 and 0.2 mg L<sup>-1</sup> and the Positive control (Table 3). The best results found for *C. labiata* about Pyridoxine were with 0.125 mg L<sup>-1</sup> for all variables evaluated. The dry mass was statistically different from all others. For the length variables (root and shoot), it did not differ from the Positive control and the 0.25 mg L<sup>-1</sup> concentration. For the number variables (shoots and roots), the 0.125 mg L<sup>-1</sup> concentration did not differ from the Negative control, Positive control and the 0.25 mg L<sup>-1</sup> concentration, as can be seen in table 3.

The use of Nicotinamide for *C. labiata*, in the variables of length and number of roots, as well as dry matter, did not present significant differences. For shoot length, the Negative and Positive controls and the 0.25, 0.5 and 1 mg L<sup>-1</sup> concentrations presented the best results. The number of shoots did not show

Table 1 - Treatments and concentrations of vitamins of B Complex (Thiamine - B1, Nicotinamide - B3 and Pyridoxine - B6) applied to *Cattleya labiata*, *C. walkeriana* and *C. brevicaulis*.

| Vitamin (mg L <sup>-1</sup> ) | Treatments |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-------------------------------|------------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                               | N          | P    | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
| B1                            | 0.00       | 0.05 | 0.03 | 0.05 | 0.10 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| B3                            | 0.00       | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.25 | 0.50 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| B6                            | 0.00       | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.25 | 0.50 | 1.00 |

Table 2 - Thiamine, Pyridoxine and Nicotinamide concentration effects on fresh mass (g) increase of *Cattleya labiata* during 120 days.

| Concentration (mg L <sup>-1</sup> ) | Time (days)       |                   |                   |                   |                                 |                  | Regression      | R <sup>2</sup>      |
|-------------------------------------|-------------------|-------------------|-------------------|-------------------|---------------------------------|------------------|-----------------|---------------------|
|                                     | 0                 | 30                | 60                | 90                | 120                             |                  |                 |                     |
| Negative control                    | 0.0197 <b>aaa</b> | 0.0331 <b>aaa</b> | 0.0424 <b>aaa</b> | 0.0645 <b>aba</b> | 0.1040 <b>acb</b>               | L <sub>1</sub>   |                 | 0.920 <sup>**</sup> |
| Thiamine                            | 0.025             | 0.0197 <b>a</b>   | 0.0210 <b>a</b>   | 0.0382 <b>a</b>   | 0.0577 <b>a</b>                 | 0.0832 <b>a</b>  | L <sub>2</sub>  | 0.992 <sup>**</sup> |
|                                     | 0.05              | 0.0197 <b>a</b>   | 0.0217 <b>a</b>   | 0.0446 <b>a</b>   | 0.0758 <b>a</b>                 | 0.1136 <b>a</b>  | L <sub>3</sub>  | 0.967 <sup>**</sup> |
|                                     | 0.1               | 0.0197 <b>a</b>   | 0.0210 <b>a</b>   | 0.0341 <b>a</b>   | 0.0604 <b>a</b>                 | 0.0894 <b>a</b>  | L <sub>4</sub>  | 0.910 <sup>*</sup>  |
|                                     | 0.2               | 0.0197 <b>a</b>   | 0.0222 <b>a</b>   | 0.0366 <b>a</b>   | 0.0664 <b>a</b>                 | 0.0955 <b>a</b>  | L <sub>5</sub>  | 0.941 <sup>**</sup> |
| CV (%)                              | 30.24             |                   |                   |                   |                                 |                  |                 |                     |
| Pyridoxine                          | 0.125             | 0.0197 <b>a</b>   | 0.0547 <b>a</b>   | 0.0926 <b>a</b>   | 0.1395 <b>a</b>                 | 0.1928 <b>a</b>  | L <sub>6</sub>  | 0.991 <sup>**</sup> |
|                                     | 0.25              | 0.0197 <b>a</b>   | 0.0361 <b>a</b>   | 0.0517 <b>a</b>   | 0.0780 <b>ab</b>                | 0.1143 <b>ab</b> | L <sub>7</sub>  | 0.963 <sup>**</sup> |
|                                     | 0.5               | 0.0197 <b>a</b>   | 0.0190 <b>a</b>   | 0.0295 <b>a</b>   | 0.0462 <b>ab</b>                | 0.0660 <b>bc</b> | L <sub>8</sub>  | 0.88 <sup>**</sup>  |
|                                     | 1.0               | 0.0197 <b>a</b>   | 0.0217 <b>a</b>   | 0.0343 <b>a</b>   | 0.0534 <b>ab</b>                | 0.0701 <b>c</b>  | L <sub>9</sub>  | 0.925 <sup>**</sup> |
| CV (%)                              | 38.47             |                   |                   |                   |                                 |                  |                 |                     |
| Nicotinamide                        | 0.125             | 0.0197 <b>a</b>   | 0.0338 <b>a</b>   | 0.0605 <b>a</b>   | 0.0823 <b>a</b>                 | 0.1101 <b>ab</b> | L <sub>10</sub> | 0.954 <sup>**</sup> |
|                                     | 0.25              | 0.0197 <b>a</b>   | 0.0217 <b>a</b>   | 0.0446 <b>a</b>   | 0.0901 <b>a</b>                 | 0.1314 <b>a</b>  | Q <sub>1</sub>  | 0.998 <sup>*</sup>  |
|                                     | 0.5               | 0.0197 <b>a</b>   | 0.0216 <b>a</b>   | 0.0360 <b>a</b>   | 0.0641 <b>a</b>                 | 0.0951 <b>c</b>  | Q <sub>2</sub>  | 0.998 <sup>*</sup>  |
|                                     | 1.0               | 0.0197 <b>a</b>   | 0.0222 <b>a</b>   | 0.0366 <b>a</b>   | 0.0664 <b>a</b>                 | 0.0955 <b>bc</b> | Q <sub>3</sub>  | 0.998 <sup>*</sup>  |
| CV (%)                              | 37.76             |                   |                   |                   |                                 |                  |                 |                     |
| Positive control                    | 0.0197 <b>aaa</b> | 0.0275 <b>aaa</b> | 0.0483 <b>aaa</b> | 0.0883 <b>aaa</b> | 0.1210 <b>aaaba</b><br><b>b</b> |                  | Q <sub>4</sub>  | 0.989 <sup>*</sup>  |

Different letters in the column: normal type = Thiamine; italic = Pyridoxine and bold = Nicotinamide. Regression: L = Linear; Q = Quadratic. L<sub>1</sub> = 0.000667x - 0.0127; L<sub>2</sub> = 0.00144x - 0.0136; L<sub>3</sub> = 0.00771 - 0.0137; L<sub>4</sub> = 0.0121x + 0.0003; L<sub>5</sub> = 0.00044x + 0.0133; L<sub>6</sub> = 0.0431x - 0.0294; L<sub>7</sub> = 0.0231x - 0.00094; L<sub>8</sub> = 0.0121x + 0.0001; L<sub>9</sub> = 0.013x + 0.00001; L<sub>10</sub> = 0.000529x + 0.0093; Q<sub>1</sub> = 0.000006x<sup>2</sup> - 0.00015x + 0.0144; Q<sub>2</sub> = 0.000005x<sup>2</sup> - 0.00068x + 0.0152; Q<sub>3</sub> = 0.00005x<sup>2</sup> - 0.00011x + 0.0150; Q<sub>4</sub> = 0.000006x<sup>2</sup> + 0.0024x + 0.0137. Means followed by equal letters are not different by Tukey's test (P < 0.05). Regression significance (\*P < 0.05, \*\*P < 0.01).

statistical differences between the Negative and Positive controls and the 0.25 mg L<sup>-1</sup> concentration (Table 3).

Table 4 shows the results regarding the fresh mass in grams of *Cattleya walkeriana* seedlings. These presented a greater accumulation of fresh mass at the standard concentration of Thiamine, isolated or in combination with Pyridoxine and Nicotinamide in the positive control.

For Pyridoxine, the best results were observed at the concentration of 1 mg L<sup>-1</sup>, but did not differ from 0.5 mg L<sup>-1</sup> or the Positive control. Nicotinamide exhibited similar performance across nearly all treatments, with the concentrations of 0.125, 0.25, 1.0 mg L<sup>-1</sup> and the Positive control showed more satisfactory results at the end of 120 days. There was a cumulative and increasing response over the time studied, for all treatments, which can be observed in the regressions, which were all significant (Table 4).

In *C. walkeriana* seedlings, at any Thiamine concentration, there were no significant differences for the number of shoots and roots. For root length, the concentration of 0.05 mg L<sup>-1</sup> of

Thiamine was statistically superior to the negative control, and in the growth of the aerial part, was statistically different in the Negative control and the highest concentration (0.2 mg L<sup>-1</sup>). For the dry mass variable, we highlight that the Negative control and the Positive control differed statistically (Table 5).

Pyridoxine, at different concentrations, did not affect the length of the aerial part, dry mass, or number of shoots in *C. walkeriana* seedlings (Table 5). In terms of root length, the lowest concentration (0.125 mg L<sup>-1</sup>) as well as the negative control resulted in lower values than the other levels tested. For the number of roots, twice the concentration commonly used (0.5 mg L<sup>-1</sup>) differed from both the Negative control and the Positive control.

The different concentrations of Nicotinamide in *C. walkeriana* seedlings showed the same effect on the length of the aerial part. For root length, the negative control was the least satisfactory treatment. For the number of shoots, the positive control was statistically different from any concentration of Nicotinamide. For dry mass, the positive control and the highest concentration (1 mg L<sup>-1</sup>) were statistically superior only to the negative control (Table 5).

Table 3 - Effect of different concentrations of Thiamine, Pyridoxine and Nicotinamide on the variables length of the largest root and shoot, number of shoots and roots and dry mass of *Cattleya labiata* cultivated for 120 days.

|                                              | -----Root-----      | -----Shoot----- | ---Root lenght--- | ---Shoot lenght--- | -----Dry mass---- |
|----------------------------------------------|---------------------|-----------------|-------------------|--------------------|-------------------|
|                                              | ------(number)----- |                 | ------(mm)-----   |                    | ------(g)-----    |
| -----Thiamine (mg L <sup>-1</sup> )-----     |                     |                 |                   |                    |                   |
| Negative control                             | 3.43a               | 3.93a           | 15.33a            | 6.80b              | 0.0097a           |
| 0.025                                        | 3.57a               | 3.47a           | 16.33a            | 7.93ab             | 0.0096a           |
| 0.05                                         | 3.23a               | 3.23a           | 17.43a            | 9.36a              | 0.0116a           |
| 0.1                                          | 3.53a               | 3.87a           | 18.20a            | 8.90ab             | 0.0115a           |
| 0.2                                          | 4.33a               | 3.37a           | 20.90a            | 8.50ab             | 0.0132a           |
| Positive control                             | 3.46a               | 3.50a           | 18.77a            | 8.13ab             | 0.0113a           |
| -----Pyridoxine (mg L <sup>-1</sup> )-----   |                     |                 |                   |                    |                   |
| Negative control                             | 3.43abc             | 3.93ab          | 15.33bc           | 6.80c              | 0.0097b           |
| 0.125                                        | 4.90a               | 4.60a           | 22.07a            | 9.33a              | 0.0179a           |
| 0.25                                         | 4.05ab              | 3.87ab          | 18.81ab           | 9.08ab             | 0.0122b           |
| 0.5                                          | 2.32c               | 2.60b           | 15.56bc           | 7.48bc             | 0.0070b           |
| 1                                            | 2.93bc              | 2.77b           | 13.17c            | 7.00c              | 0.0076b           |
| Positive control                             | 3.47abc             | 3.50ab          | 18.77ab           | 8.13abc            | 0.0113b           |
| -----Nicotinamide (mg L <sup>-1</sup> )----- |                     |                 |                   |                    |                   |
| Negative control                             | 3.43a               | 3.93a           | 15.33a            | 6.80ab             | 0.0097a           |
| 0.125                                        | 3.47a               | 2.53b           | 15.87a            | 6.33b              | 0.0079a           |
| 0.25                                         | 3.27a               | 2.97ab          | 17.83a            | 7.07ab             | 0.0111a           |
| 0.5                                          | 2.90a               | 2.63b           | 15.60a            | 6.73ab             | 0.0082a           |
| 1                                            | 3.24a               | 2.56b           | 18.04a            | 7.08ab             | 0.0099a           |
| Positive control                             | 3.47a               | 3.50a           | 18.77a            | 8.13a              | 0.0113a           |

Means followed by the same vertical letter do not differ according to the Tukey test at 5% probability.

For *C. brevicaulis* seedlings, the greatest accumulation of fresh mass in the treatments with Thiamine was observed at concentrations of 0.1 and 0.2 mg L<sup>-1</sup>, not differing from other concentrations (Table 6). The negative control presented the lowest results. For Pyridoxine, the differences were more visible after 60 days. Numerically, the treatment with the lowest treatment (0.125 mg L<sup>-1</sup>) stood out, but did not differ from the other treatments (Table 6). Once again, the Negative control had the worst performance. Considering Nicotinamide alone at 120 days, the concentrations of 0.125 and 0.25 mg L<sup>-1</sup> did not differ from each other or the concentration of 0.5 mg L<sup>-1</sup>. Still, they differed from the others, especially from the negative control.

There was an increasing and cumulative response over time for all treatments, which can be observed by linear or quadratic regressions, which were all significant (Table 6). The use of different concentrations of Thiamine did not affect the root length of *C. brevicaulis* seedlings. For the shoot length variable, the Negative control and the concentrations of 0.05 and 0.2 mg L<sup>-1</sup> did not differ from each other, and the absence of Thiamine was

advantageous for shoot growth. For the number of roots, the Positive control was statistically inferior to all other treatments, but for the number of shoots, only the highest concentration differed negatively. Considering the dry mass variable, the concentrations of 0.2, 0.1 mg L<sup>-1</sup> and the Negative control did not differ from each other (Table 7).

The use of different concentrations of Pyridoxine did not affect the accumulation of dry mass in *C. brevicaulis* seedlings. The Positive control had a lower result for the length of the largest root than the other treatments. For shoot length, the concentration of 0.125 mg L<sup>-1</sup> and the Negative control were statistically different from the concentration of 0.25 mg L<sup>-1</sup>. For the variable number of roots, the concentration of 1.0 mg L<sup>-1</sup> differed only from the control and the concentration of 0.25 mg L<sup>-1</sup>. For the variable number of shoots, the Negative control, Positive control and the concentration of 0.125 mg L<sup>-1</sup> differed from each other.

*C. brevicaulis* seedlings exposed to different concentrations of Nicotinamide did not differ statistically for shoot length and dry mass. The isolated use of Nicotinamide, at any concentration,

Table 4 - Thiamine, Pyridoxine and Nicotinamide concentration effects on fresh mass (g) increase of *Cattleya walkeriana* during 120 days.

| Concentration (mg L <sup>-1</sup> ) | Time (days) |            |           |             |           |                 | Regression | R <sup>2</sup> |
|-------------------------------------|-------------|------------|-----------|-------------|-----------|-----------------|------------|----------------|
|                                     | 0           | 30         | 60        | 90          | 120       |                 |            |                |
| Negative control                    | 0.0120aaa   | 0.0182 aaa | 0.0385aaa | 0.0512bba   | 0.1339bbb | Q <sub>1</sub>  |            | 0.953**        |
| Thiamine                            | 0.025       | 0.0120a    | 0.0268a   | 0.0397a     | 0.0690ab  | Q <sub>2</sub>  |            | 0.978**        |
|                                     | 0.05        | 0.0120a    | 0.0310a   | 0.0524a     | 0.0923ab  | Q <sub>3</sub>  |            | 0.982**        |
|                                     | 0.1         | 0.0120a    | 0.0283a   | 0.0452a     | 0.0694a   | L <sub>1</sub>  |            | 0.917*         |
|                                     | 0.2         | 0.0120a    | 0.0269a   | 0.0457a     | 0.0559ab  | L <sub>2</sub>  |            | 0.881**        |
| CV (%)                              | 40.49       |            |           |             |           |                 |            |                |
| Pyridoxine                          | 0.125       | 0.0120a    | 0.0226a   | 0.0631a     | 0.1027a   | Q <sub>4</sub>  |            | 0.972*         |
|                                     | 0.25        | 0.0120a    | 0.0248a   | 0.0352a     | 0.0622ab  | Q <sub>5</sub>  |            | 0.977**        |
|                                     | 0.5         | 0.0120a    | 0.0251a   | 0.0468a     | 0.0824ab  | Q <sub>6</sub>  |            | 0.984**        |
|                                     | 1.0         | 0.0120a    | 0.0327a   | 0.0511a     | 0.0869ab  | Q <sub>6</sub>  |            | 0.975**        |
| CV (%)                              | 38.49       |            |           |             |           |                 |            |                |
| Nicotinamide                        | 0.125       | 0.0120a    | 0.0357a   | 0.0509a     | 0.0737a   | Q <sub>7</sub>  |            | 0.975**        |
|                                     | 0.25        | 0.0120a    | 0.0278a   | 0.0425a     | 0.0737a   | Q <sub>8</sub>  |            | 0.973**        |
|                                     | 0.5         | 0.0120a    | 0.0291a   | 0.0490a     | 0.0838a   | L <sub>4</sub>  |            | 0.943**        |
|                                     | 1.0         | 0.0120a    | 0.0261a   | 0.0488a     | 0.0716a   | Q <sub>9</sub>  |            | 0.968**        |
| CV (%)                              | 35.04       |            |           |             |           |                 |            |                |
| Positive control                    | 0.0120aaa   | 0.0237aaa  | 0.0357aaa | 0.0682ababa | 0.1920aaa | Q <sub>10</sub> |            | 0.960**        |

Different letters in the column: normal type = Thiamine; italic = Pyridoxine and bold = Nicotinamide. Regression: L = Linear; Q = Quadratic. Q<sub>1</sub> = 0.000012x<sup>2</sup> - 0.0005x + 0.0162; Q<sub>2</sub> = 0.000011x<sup>2</sup> - 0.0003x + 0.0167; Q<sub>3</sub> = 0.000015x<sup>2</sup> - 0.00035x + 0.0177; L<sub>1</sub> = 0.0009x + 0.0023; L<sub>2</sub> = 0.0008x + 0.0039; L<sub>3</sub> = 0.0011x + 0.0003; Q<sub>4</sub> = 0.000010x<sup>2</sup> - 0.00033x + 0.0658; Q<sub>5</sub> = 0.000014x<sup>2</sup> - 0.00039x + 0.0165; Q<sub>6</sub> = 0.000014x<sup>2</sup> - 0.00035x + 0.0184; Q<sub>7</sub> = 0.000011x<sup>2</sup> - 0.00017x + 0.0188; Q<sub>8</sub> = 0.000014x<sup>2</sup> - 0.00044x + 0.0179; L<sub>4</sub> = 0.0010x + 0.0126; Q<sub>9</sub> = 0.000012x<sup>2</sup> - 0.00033x + 0.0170; Q<sub>10</sub> = 0.000019x<sup>2</sup> - 0.0010x + 0.0204. Means followed by equal letters are not different by Tukey's test (P < 0.05). Regression significance (\*P < 0.05, \*\*P < 0.01).

was statistically different from the Positive control for the length of the largest root and the number of roots. For the number of shoots, the concentration of 0.25 mg L<sup>-1</sup> was statistically different from 1.0 mg L<sup>-1</sup>, but did not differ from the others (Table 7).

## DISCUSSION

The external addition of Thiamine can provide a greater ability to tolerate abiotic stresses by increasing osmotic tolerance, alleviating dehydration, and improving the growth under salinity stress. Thiamine priming can induce plant's ability to mount faster and stronger defense responses upon subsequent exposure to stress or pathogens, similar to the effect of abscisic acid (LI et al., 2022) and increasing the capacity to supply NADH or NADPH (TUNC-OZDEMIR et al., 2009), in this case also linked to a greater supply of Nicotinamide.

The data obtained in this study showed that the effect of different concentrations of Thiamine was reduced, and the concentration used (0.05 mg L<sup>-1</sup>) (half the concentration used in the MS medium) for orchid cultivation was among the most suitable for the accumulation of fresh mass in the three species

studied. For the counting variables (number of shoots and roots), only *C. brevicaulis* showed a negative response to the highest concentration of Thiamine (0.2 mg L<sup>-1</sup>). Nevertheless, for the variables of length and dry mass, there were different responses from each species, and except for *C. brevicaulis*, for dry mass, there was a tendency to maintain the concentration of 0.05 mg L<sup>-1</sup> as one of the most effective.

The addition of this vitamin has been proven to increase the plant height in *Dendrobium* sp (LATIF et al., 2020), leaf span and root number in *Phalaenopsis* (HANDINI et al., 2023). However, its use in tissue culture is not completely established, as it can lead to the induction or inhibition of callus, root and embryo growth (ABRAHAMIAN & KANTHARAJAH, 2011). According to FITZPATRICK & CHAPMAN (2020), vitamin B1 or Thiamine is associated with several physiological factors in living beings, acting as a cofactor in enzymatic reactions for energy production, contributing to tolerance against environmental stressors, influencing defense mechanisms, vital for growth and development; overall, it is central for many metabolic pathways, and this could be an explanation for the results obtained here.

Table 5 - Effect of different concentrations of Thiamine, Pyridoxine and Nicotinamide on the variables length of the largest root and shoot, number of shoots and roots and dry mass of *Cattleya walkeriana* cultivated for 120 days.

|                                    | -----Root-----      | -----Shoot----- | ---Root lenght--- | ---Shoot lenght--- | -----Dry mass----- |
|------------------------------------|---------------------|-----------------|-------------------|--------------------|--------------------|
|                                    | ------(number)----- |                 | ------(mm)-----   |                    | ------(g)-----     |
| Thiamine (mg L <sup>-1</sup> )     |                     |                 |                   |                    |                    |
| Negative control                   | 2.67a               | 3.30a           | 9.72b             | 6.90bc             | 0.0109b            |
| 0.025                              | 3.13a               | 4.23a           | 12.12ab           | 8.63ab             | 0.0147ab           |
| 0.05                               | 4.15a               | 3.16a           | 13.79a            | 8.94a              | 0.0143ab           |
| 0.1                                | 3.57a               | 3.60a           | 11.30ab           | 8.00abc            | 0.0130ab           |
| 0.2                                | 3.93a               | 3.67a           | 11.80ab           | 6.30c              | 0.0123ab           |
| Positive control                   | 2.95a               | 5.36a           | 11.43ab           | 7.13ab             | 0.0177a            |
| Piridoxine (mg L <sup>-1</sup> )   |                     |                 |                   |                    |                    |
| Negative control                   | 2.67c               | 3.30a           | 9.72c             | 6.90a              | 0.0109a            |
| 0.125                              | 3.96abc             | 5.60a           | 11.08bc           | 7.32a              | 0.0208a            |
| 0.25                               | 4.73ab              | 2.83a           | 13.57ab           | 7.40a              | 0.0142a            |
| 0.5                                | 4.87a               | 4.97a           | 14.27a            | 8.50a              | 0.0164a            |
| 1                                  | 4.57abc             | 4.53a           | 11.83abc          | 7.13a              | 0.0180a            |
| Positive control                   | 2.95bc              | 5.36a           | 11.43abc          | 7.13a              | 0.0177a            |
| Nicotinamide (mg L <sup>-1</sup> ) |                     |                 |                   |                    |                    |
| Negative control                   | 2.67b               | 3.30b           | 9.72b             | 6.90a              | 0.0100b            |
| 0.125                              | 4.63ab              | 3.37b           | 13.97a            | 7.03a              | 0.0150ab           |
| 0.25                               | 4.90a               | 3.40b           | 13.53a            | 7.60a              | 0.0133ab           |
| 0.5                                | 4.13ab              | 3.30b           | 14.10a            | 7.20a              | 0.0133ab           |
| 1                                  | 4.70ab              | 3.43b           | 12.93ab           | 6.47a              | 0.0183a            |
| Positive control                   | 2.95ab              | 5.36a           | 11.43ab           | 7.13a              | 0.0200a            |

Means followed by the same vertical letter do not differ according to the Tukey test at 5% probability.

Table 6 - Thiamine, Pyridoxine and Nicotinamide concentration effects on fresh mass (g) increase of *Cattleya brevicaulis* during 120 days.

| Concentration (mg L <sup>-1</sup> ) | Time (days) |              |           |              |           |                |  |                |
|-------------------------------------|-------------|--------------|-----------|--------------|-----------|----------------|--|----------------|
|                                     | 0           | 30           | 60        | 90           | 120       | Regression     |  | R <sup>2</sup> |
| Negative control                    | 0.0913aaa   | 0.0302bbb    | 0.0333ccb | 0.0367ccb    | 0.0478cd  | Q <sub>1</sub> |  | 0.845**        |
| Thiamine                            | 0.025       | 0.0913a      | 0.0667ab  | 0.0871b      | 0.1131ab  | Q <sub>2</sub> |  | 0.941*         |
|                                     | 0.05        | 0.0913a      | 0.0729ab  | 0.1095ab     | 0.1222ab  | Q <sub>3</sub> |  | 0.942*         |
|                                     | 0.1         | 0.0913a      | 0.1000a   | 0.1418a      | 0.1448ab  | L <sub>1</sub> |  | 0.929**        |
|                                     | 0.2         | 0.0913a      | 0.1085a   | 0.1488a      | 0.1575a   | L <sub>2</sub> |  | 0.962**        |
| CV (%)                              | 27.86       |              |           |              |           |                |  |                |
| Piridoxine                          | 0.125       | 0.0913a      | 0.0792ab  | 0.1607a      | 0.1676a   | L <sub>3</sub> |  | 0.876**        |
|                                     | 0.25        | 0.0913a      | 0.0559ab  | 0.0966abc    | 0.1124ab  | L <sub>4</sub> |  | 0.597**        |
|                                     | 0.5         | 0.0913a      | 0.0458ab  | 0.0743bc     | 0.0794bc  | Q <sub>4</sub> |  | 0.734*         |
|                                     | 1.0         | 0.0913a      | 0.1061a   | 0.1330ab     | 0.1394ab  | L <sub>5</sub> |  | 0.976**        |
| CV (%)                              | 41.49       |              |           |              |           |                |  |                |
| Nicotinamide                        | 0.125       | 0.0913a      | 0.0700ab  | 0.1133a      | 0.1333a   | Q <sub>5</sub> |  | 0.965**        |
|                                     | 0.25        | 0.0913a      | 0.0683ab  | 0.1250a      | 0.1417a   | Q <sub>6</sub> |  | 0.949**        |
|                                     | 0.5         | 0.0913a      | 0.0567ab  | 0.0883a      | 0.1067a   | Q <sub>7</sub> |  | 0.964**        |
|                                     | 1.0         | 0.0913a      | 0.0817a   | 0.1017a      | 0.1117a   | Q <sub>8</sub> |  | 0.968**        |
| CV (%)                              | 27.75       |              |           |              |           |                |  |                |
| Positive control                    | 0.0913aaa   | 0.0745ababab | 0.0863    | 0.1016ababca | 0.1325bbc | Q <sub>9</sub> |  | 0.960**        |

Different letters in the column: normal type = Thiamine; italic = Pyridoxine and bold = Nicotinamide. Regression: L = Linear; Q = Quadratic. Q<sub>1</sub> = 0.000012x<sup>2</sup> - 0.0005x + 0.0162; Q<sub>2</sub> = 0.000009x<sup>2</sup> - 0.00058x + 0.0866; Q<sub>3</sub> = 0.000009x<sup>2</sup> - 0.00034x + 0.0873; L<sub>1</sub> = 0.00082x + 0.0847; L<sub>2</sub> = 0.00092x + 0.0870; L<sub>3</sub> = 0.0011x + 0.0761; L<sub>4</sub> = 0.00046x + 0.0701; Q<sub>4</sub> = 0.00001x<sup>2</sup> - 0.00096x + 0.0842; L<sub>5</sub> = 0.00060x + 0.0907; Q<sub>5</sub> = 0.000014x<sup>2</sup> - 0.00067x + 0.0886; Q<sub>6</sub> = 0.000014x<sup>2</sup> - 0.00056x + 0.0874; Q<sub>7</sub> = 0.000017x<sup>2</sup> - 0.00128x + 0.0890; Q<sub>8</sub> = 0.000008x<sup>2</sup> - 0.00044x + 0.0911; Q<sub>9</sub> = 0.000008x<sup>2</sup> - 0.00058x + 0.0895. Means followed by equal letters are not different by Tukey's test (P < 0.05), Regression significance (\*P < 0.05, \*\*P < 0.01).

Pyridoxine, specifically in its active form, pyridoxal 5'-phosphate, is a crucial cofactor for over 140 biochemical reactions in plants (ÉVA HIDEG et al., 2020). This vitamin participates in numerous biochemical reactions in plants, acts as an antioxidant, protecting from ROS during stress, is required for nitrogen metabolism, and if deficient, can result in reduced shoot, root, and leaf development, as well as delayed flowering (COLINAS & FITZPATRICK, 2016).

In the present study, the data indicated that lower concentrations (absence or 0.125 mg L<sup>-1</sup>) than those routinely used in the UNOESTE laboratory (0.25 mg L<sup>-1</sup>) may be more efficient, considering not only the accumulation of fresh matter but also all the variables analyzed. This may indicate that these plants require a very low concentration of this vitamin in the medium. Because the cultivation conditions were similar for all treatments, there was no increase in the root and aerial part length, number of roots or shoots, or dry mass. Similar effect was found for *Coelogyne rochussenii*, where different concentrations of Pyridoxine did not significantly

affect the number and height of shoots, number of leaves, number and length of roots (HABIBAH et al., 2023).

Nicotinamide has been described as a precursor of NAD and NADP, which actively participate in cellular oxidation-reduction reactions and as energy carriers (ABRAHAMIAN & KANTHARAJAH, 2011; MIRET & MUNNÉ-BOSCH, 2014) or as modulating saline stress tolerance (ABDELHAMID et al., 2013; SADAK et al., 2010).

The exogenous administration of nicotinamide activates the physiological mechanisms within the plant (COLLA et al., 2021) and enhances both vegetative and reproductive growth as a result of an increased synthesis of photoassimilates (DONG et al., 2015), resulting in stronger vegetative and reproductive development. LIMA et al. (2024) observed in soybean culture that the utilisation of nicotinamide influences overall productivity, enhancing it considerably in regions adversely affected by water stress.

Furthermore, nicotinamide's positive effects on chloroplasts could promote the accumulation of

Table 7 - Effect of different concentrations of Thiamine, Pyridoxine and Nicotinamide on the variables length of the largest root and shoot, number of shoots and roots and dry mass of *Cattleya brevicaulis* cultivated for 120 days.

|                                              | -----Root-----      | -----Shoot----- | ----Root lenght---- | ----Shoot lenght---- | -----Dry mass----- |
|----------------------------------------------|---------------------|-----------------|---------------------|----------------------|--------------------|
|                                              | ------(number)----- |                 | ------(mm)-----     |                      | ------(g)-----     |
| -----Thyamine (mg L <sup>-1</sup> )-----     |                     |                 |                     |                      |                    |
| Negative control                             | 4.00ab              | 5.73a           | 10.57a              | 27.23a               | 0.0233ab           |
| 0.025                                        | 3.67ab              | 5.00a           | 10.80a              | 18.00bc              | 0.0167b            |
| 0.05                                         | 3.37ab              | 5.60a           | 9.53a               | 20.07abc             | 0.0200b            |
| 0.1                                          | 3.60ab              | 4.80ab          | 12.40a              | 17.37c               | 0.0217ab           |
| 0.2                                          | 4.60a               | 3.43b           | 12.57a              | 25.77ab              | 0.0317a            |
| Positive control                             | 3.00b               | 4.90a           | 9.20a               | 17.07c               | 0.0200b            |
| -----Piridoxine (mg L <sup>-1</sup> )-----   |                     |                 |                     |                      |                    |
| Negative control                             | 4.00ab              | 5.73a           | 27.23ab             | 10.57a               | 0.0246a            |
| 0.125                                        | 3.47ab              | 5.00ab          | 25.87ab             | 10.23a               | 0.0245a            |
| 0.25                                         | 3.09b               | 3.63c           | 24.06ab             | 7.17b                | 0.0169a            |
| 0.5                                          | 3.27ab              | 3.06c           | 23.01ab             | 8.63ab               | 0.0149a            |
| 1                                            | 4.23a               | 3.73bc          | 33.50a              | 8.20ab               | 0.0246a            |
| Positive control                             | 3.00b               | 4.90ab          | 17.07b              | 9.20ab               | 0.0197a            |
| -----Nicotinamide (mg L <sup>-1</sup> )----- |                     |                 |                     |                      |                    |
| Negative control                             | 4.00ab              | 5.73ab          | 27.23a              | 10.57a               | 0.0246a            |
| 0.125                                        | 5.59a               | 5.71ab          | 29.96a              | 8.69a                | 0.0240a            |
| 0.25                                         | 4.90a               | 6.33a           | 27.23a              | 8.63a                | 0.0230a            |
| 0.5                                          | 5.53a               | 4.63ab          | 28.13a              | 9.43a                | 0.0241a            |
| 1                                            | 4.94a               | 4.33b           | 31.50a              | 8.52a                | 0.0246a            |
| Positive control                             | 3.00b               | 4.90ab          | 17.07b              | 9.20a                | 0.0198a            |

Means followed by the same vertical letter do not differ according to the Tukey test at 5% probability.

nitrogenous compounds, contributing to increased grain mass during maturation (BERNARDO et al., 2024).

Nicotinamide has been shown to enhance the growth of orchid seedlings across various ages; moreover, it is often added to orchid growth media to improve germination and seedling development, suggesting a key role in early growth stages (ARDITTI, 1967).

Thus, an increase in the concentration of nicotinamide could increase metabolism and accelerate plant growth and development; however, the data demonstrate, in general, that high concentrations are not responsive or do not differ from lower ones, which may be in agreement with NEILL et al. (2003) where nicotinamide inhibits stomatal closure induced by ABA or nitrous oxide (NO), which could increase the respiration rate and decrease plant growth. If these compounds are used as energy carriers, these molecules are regenerable during the various metabolic cycles, corroborating the data obtained here, that is, that small quantities would be necessary for the growth and development of these plants.

## CONCLUSION

The complex B vitamins, Thiamine, Nicotinamide and Pyridoxine exhibited isolated effects in orchid seedling growth. It is recommended to reduce the Thiamine concentration to 0.025 mg L<sup>-1</sup>, decrease the Pyridoxine concentration to 0.125 mg L<sup>-1</sup>, and no Nicotinamide to the medium for the species studied.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

## DATA AVAILABILITY STATEMENT

Data will be available upon request to the corresponding author.

## DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

The software Grammarly was used to maintain the paper's readability. The software Zotero is used to do the references and citations. No other kind of AI was used for any other purpose.

## REFERENCES

- ABBASZADEH, S. M. et al. An effective nutrient media for asymbiotic seed germination and *in vitro* seedling development of *Phalaenopsis* 'Bahia Blanca'. **Journal of Ornamental Plants**, v.8, n.3, p.183-192, 2018. Available from: <[http://jornamental.iaurasht.ac.ir/article\\_542585.html](http://jornamental.iaurasht.ac.ir/article_542585.html)>. Accessed: Apr. 15, 2025.
- ABDELHAMID, M. T. et al. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. **Acta Biológica Colombiana**, v.18, n.3, p.499-509, 2013. Available from: <<https://www.redalyc.org/pdf/3190/319029232009.pdf>>. Accessed: Apr. 15, 2025.
- ABRAHAMIAN, P.; KANTHARAJAH, A. Effect of vitamins on *in vitro* organogenesis of plant. **American Journal of Plant Sciences**, v.2, n.05, p.669-674, 2011. Available from: <<https://www.scirp.org/html/8740.html>>. Accessed: Apr. 15, 2025 doi: 10.4236/ajps.2011.25080.
- ARDITTI, J. Niacin Biosynthesis in Germinating *×Laeliocattleya* orchid embryos and young seedlings. **American Journal of Botany**, v.54, n.3, p.291-298, 1967. Available from: <<https://onlinelibrary.wiley.com/doi/abs/10.1002/j.1537-2197.1967.tb06923.x>>. Accessed: Apr. 14, 2025. doi: 10.1002/j.1537-2197.1967.tb06923.x.
- BERNARDO, V. F. et al. Nicotinamide and phytohormones as biostimulants in common bean. **Revista Ceres**, v.71, p.e71035, 2024. Available from: <<https://www.scielo.br/j/rceres/a/T5p8XpVmSb38f7hyNHs9cpt/?lang=en>>. Accessed: Apr. 14, 2025. doi: 10.1590/0034-737X2024710035.
- COLINAS, M.; FITZPATRICK, T. B. Interaction between vitamin B6 metabolism, nitrogen metabolism and autoimmunity. **Plant Signaling & Behavior**, v.11, n.4, p.e1161876, 2016. Available from: <<https://doi.org/10.1080/15592324.2016.1161876>>. Accessed: Apr. 14, 2025. doi: 10.1080/15592324.2016.1161876.
- COLLA, R. E. S. et al. Does foliar nicotinamide application affect second crop corn (*Zea mays*)?. **Revista de la Facultad de Ciencias Agrarias UNCuyo**, v.53, p.64-70, 2021. Available from: <<https://revistas.uncu.edu.ar/ojs3/index.php/RFCA/article/view/3145>>. Accessed: Apr. 14, 2025. doi: 10.48162/rev.39.040.
- DIANTINA, S. et al. Comparative *in vitro* seed germination and seedling development in tropical and temperate epiphytic and temperate terrestrial orchids. **Plant Cell, Tissue and Organ Culture**, v.143, n.3, p.619-633, 2020. Available from: <<https://doi.org/10.1007/s11240-020-01947-7>>. Accessed: Apr. 14, 2025. doi: 10.1007/s11240-020-01947-7.
- DONG, W. et al. Enhancement of thiamin content in *Arabidopsis thaliana* by metabolic engineering. **Plant and Cell Physiology**,

- v.56, n.12, p.2285-2296, 2015. Available from: <<https://doi.org/10.1093/pcp/pcv148>>. Accessed: Apr. 14, 2025. doi: 10.1093/pcp/pcv148.
- ÉVA HIDEG, S. N. et al. Ultraviolet-B radiation exposure lowers the antioxidant capacity in the *Arabidopsis thaliana* pdx1.3-1 mutant and leads to glucosinolate biosynthesis alteration in both wild type and mutant. **Photochemical & Photobiological Sciences**, v.19, n.2, p.217-228, 2020. Available from: <<https://link.springer.com/10.1039/c9pp00342h>>. Accessed: Apr. 15, 2025. doi: 10.1039/c9pp00342h.
- FAY, M. F. Orchid conservation: how can we meet the challenges in the twenty-first century? **Botanical Studies**, v.59, n.1, p.16, 2018. Available from: <<https://doi.org/10.1186/s40529-018-0232-z>>. Accessed: Dec. 11, 2023. doi: 10.1186/s40529-018-0232-z.
- FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v.35, n.6, p.1039-1042, 2011. Available from: <<https://www.scielo.br/j/cagro/a/yjKLJXN9KysfmX6rvL93TSh/?format=html&lang=en>>. Accessed: Apr. 14, 2025. doi: 10.1590/S1413-70542011000600001.
- FITZPATRICK, T. B.; CHAPMAN, L. M. The importance of thiamine (vitamin B1) in plant health: From crop yield to biofortification. **Journal of Biological Chemistry**, v.295, n.34, p.12002-12013, 2020. Available from: <[https://www.jbc.org/article/S0021-9258\(17\)50064-2/abstract](https://www.jbc.org/article/S0021-9258(17)50064-2/abstract)>. Accessed: Apr. 14, 2025. doi: 10.1074/jbc.REV120.010918.
- FLORA DO BRASIL. **Jardim Botânico do Rio de Janeiro**. 2020. Available from: <<http://floradobrasil.jbrj.gov.br/>>. Accessed: Apr. 14, 2025.
- GNASEKARAN, P. et al. A study on the use of organic additives on the protocorm-like bodies (PLBs) growth of *Phalaenopsis violacea* orchid. **Journal of Phytology**, v.2, p.29-33, 2010. Available from: <<http://updatepublishing.com/journal/index.php/jp/article/view/2069>>. Accessed: Apr. 14, 2025.
- GOYER, A. Thiamine in plants: aspects of its metabolism and functions. **Phytochemistry**, v.71, n.14-15, p.1615-1624, 2010. Available from: <<https://doi.org/10.1016/j.phytochem.2010.06.022>>. Accessed: Apr. 14, 2025. doi: 10.1016/j.phytochem.2010.06.022.
- HANDINI, R. et al. The effect of thiamine HCL concentration variation on the seedling of *Phalaenopsis* var. Happy Valentine. **Quagga: Jurnal Pendidikan dan Biologi**, v.16, p.94-101, 2023. Available from: <<https://journal.fkip.uniku.ac.id/quagga/article/view/91>>. Accessed: Apr. 14, 2025. doi: 10.25134/quagga.v16i1.91.
- HJNER, J. A.; ARDITTI, J. Orchid mycorrhiza: vitamin production and requirements by the symbionts. **American Journal of Botany**, v.60, n.8, p.829-835, 1973. Available from: <<https://bsapubs.onlinelibrary.wiley.com/doi/abs/10.1002/j.1537-2197.1973.tb07595.x>>. Accessed: Apr. 14, 2025. doi: 10.1002/j.1537-2197.1973.tb07595.x.
- LATIF, R. A. et al. Stimulasi pertumbuhan dan perkembangan planlet anggrek (*Dendrobium* sp.) pada tahap aklimatisasi dengan pemberian vitamin B1 dan atonik. **Jurnal Ilmiah Pertanian (JIPERTA)**, v.2, n.2, p.127-134, 2020. Available from: <<https://jurnalmahasiswa.uma.ac.id/index.php/jiperta/article/view/330>>. Accessed: Apr. 14, 2025. doi: <https://doi.org/10.31289/jiperta.v2i2.330>.
- LI, W. et al. Thiamine functions as a key activator for modulating plant health and broad-spectrum tolerance in cotton. **The Plant Journal**, v.111, n.2, p.374-390, 2022. Available from: <<https://onlinelibrary.wiley.com/doi/abs/10.1111/tpj.15793>>. Accessed: Apr. 14, 2025. doi: <https://doi.org/10.1111/tpj.15793>.
- LIMA, S. F. et al. Nicotinamide as a biostimulant improves soybean growth and yield. **Open Agriculture**, v.9, p.20220259, 2024. Available from: <<https://doi.org/10.1515/opag-2022-0259>>. Accessed: Apr. 14, 2025. doi: 10.1515/opag-2022-0259.
- MACHADO NETO, N. B.; CUSTÓDIO, C. C. A medium for non-commercial sowing of orchid seed. **Selbyana**, v. 26, p.316-317, 2005. Available from: <<https://journals.flvc.org/selbyana/article/view/121437>>. Accessed: Apr. 14, 2025.
- MAHENDRAN, G. et al. Asymbiotic seed germination of *Cymbidium bicolor* Lindl. (Orchidaceae) and the influence of mycorrhizal fungus on seedling development. **Acta Physiologia Plantarum**, v.35, n.3, p.829-840, 2013. Available from: <<https://doi.org/10.1007/s11738-012-1127-3>>. Accessed: Apr. 14, 2025. doi: 10.1007/s11738-012-1127-3.
- MIRET, J. A.; MUNNÉ-BOSCH, S. Plant amino acid-derived vitamins: biosynthesis and function. **Amino Acids**, v.46, n.4, p.809-824, 2014. Available from: <<https://doi.org/10.1007/s00726-013-1653-3>>. Accessed: Apr. 14, 2025. doi: 10.1007/s00726-013-1653-3.
- MURASHIGE, T.; SKOOG, F. A Revised Medium for Rapid Growth and bioassays with Tobacco Tissue Cultures. **Physiologia Plantarum**, v.15, n.3, p.473-497, 1962. Available from: <<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1399-3054.1962.tb08052.x>>. Accessed: Apr. 14, 2025. doi: 10.1111/j.1399-3054.1962.tb08052.x.
- NEILL, S. J. et al. Nitric oxide signalling in plants. **New Phytologist**, v.159, n.1, p.11-35, 2003. Available from: <<https://nph.onlinelibrary.wiley.com/doi/10.1046/j.1469-8137.2003.00804.x>>. Accessed: Apr. 15, 2025. doi: 10.1046/j.1469-8137.2003.00804.x.
- RASMUSSEN, H. N. et al. Germination and seedling establishment in orchids: a complex of requirements. **Annals of Botany**, v.116, n.3, p.391-402, 2015. Available from: <<https://doi.org/10.1093/aob/mcv087>>. Accessed: Apr. 14, 2025. doi: 10.1093/aob/mcv087.
- RODRIGUES, D. T. et al. Cultivo *in vitro* de plântulas de orquídea em meios com diferentes concentrações de fertilizante mineral. **Revista Ceres**, v.59, p.9-15, 2012. Available from: <<https://www.scielo.br/j/rceres/a/dJMqTZv7J9k5CHBKvvNSMKL/?lang=pt>>. Accessed: Apr. 14, 2025.
- SADAK, M. S. et al. Increasing sunflower salt tolerance using nicotinamide and  $\alpha$ -tocopherol. **International Journal of Academic Research**, v.2, n.4, 2010. Available from: <[https://www.researchgate.net/profile/Maybelle-Gaballah/publication/284572937\\_Increasing\\_sunflower\\_salt\\_tolerance\\_using\\_nicotinamide\\_and\\_a-tocopherol/links/5d91c897a6fdcc2554a6b184/Increasing-sunflower-salt](https://www.researchgate.net/profile/Maybelle-Gaballah/publication/284572937_Increasing_sunflower_salt_tolerance_using_nicotinamide_and_a-tocopherol/links/5d91c897a6fdcc2554a6b184/Increasing-sunflower-salt)>.

tolerance-using-nicotinamide-and-a-tocopherol.pdf>. Accessed: Apr. 15, 2025.

SEATON, P.; RAMSAY, M. **Growing orchids from seed**. Royal Botanic Gardens, Kew, 2005.

TUNC-OZDEMIR, M. et al. Thiamine confers enhanced tolerance to oxidative stress in *Arabidopsis*. **Plant Physiology**, v.151, n.1, p.421-432, 2009. Available from: <<https://academic.oup.com/plphys/article-abstract/151/1/421/6108522>>. Accessed: Apr. 15, 2025. doi: 10.1104/pp.109.140046.

VANDERSCHUREN, H. et al. Strategies for vitamin B6 biofortification of plants: a dual role as a micronutrient Apr. 15, 2025 and a stress protectant. **Frontiers in Plant Science**, v.4, p.143, 2013. Available from: <<https://www.frontiersin.org/journals/plant->

[science/articles/10.3389/fpls.2013.00143/full](https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2013.00143/full)>. Accessed: Apr. 15, 2025. doi: 0.3389/fpls.2013.00143/full.

VILLA, F. et al. Micropropagação de híbridos de orquídea em meio Knudson com adição de vitaminas do meio MS, benzilaminopurina e carvão ativado. **Semina: Ciências Agrárias**, v.35, n.2, p.683-694, 2014. Available from: <<https://ojs.uel.br/revistas/uel/index.php/semagrarias/article/view/13286>>. Accessed: Apr. 14, 2025. doi: 10.5433/1679-0359.2014v35n2p683.

YAM, T. W.; ARDITTI, J. History of orchid propagation: a mirror of the history of biotechnology. **Plant Biotechnology Reports**, v.3, n.1, p.1-56, 2009. Available from: <<http://link.springer.com/10.1007/s11816-008-0066-3>>. Accessed: Oct. 11, 2018. doi: 10.1007/s11816-008-0066-3.