EFFECT OF GIBBERELLIC ACID AND TEMPERATURE ON GERMINATION OF *Vitex montevidensis* Cham.

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ABSTRACT: This work aims to evaluate treatments that may help improve speed and uniformity of germination in fruit tree species *tarumã*. Data were obtained from pyrenes (stones) of ripe fruits which in turn were picked from adult trees in the municipality of Vera Cruz do Oeste-PR. Treatments consisted of applying GA_3 solutions at various concentrations (zero, 50 mg L^{-1}, 100 mg L^{-1} or 200 mg L^{-1}) to pyrenes via immersion for 47 hours, and thermoperiods of alternate 20ºC and 30ºC (8 hours at 20ºC in light conditions, and 16 hours at 30ºC in dark conditions) and constant 30ºC with the same photoperiod. The combination of alternate temperatures of 20ºC and 30ºC with a 200 mg L^{-1} GA_3 solution resulted in an increase both in germination percentage, from 19.2% to 56.2%, and in the germination speed index, from 0.19 to 3.12, with resulting normal, uniform seedlings.

Key words: Pyrenes, imbibition curve, tarumã, thermoperiod.

EFEITO DO ÁCIDO GIBERÉLICO E DA TEMPERATURA NA GERMINAÇÃO DE *Vitex montevidensis* Cham.

***RESUMO**: Objetivou-se, com este trabalho, avaliar tratamentos que proporcionem maior rapidez e uniformidade na germinação de tarumã. Os dados foram obtidos de pirênios extraídos de frutos maduros coletados em árvores adultas no município de Vera Cruz do Oeste, PR. Nos tratamentos,utilizaram-se soluções com concentrações de GA_3 (zero, 50, 100, ou 200 mg L^{-1}) aplicadas aos pirênios por imersão, durante 47 horas, e termoperíodos de 20 e 30 ºC alternados (8 horas a 20 ºC no claro, e 16 horas a 30 ºC no escuro) e 30 ºC constante com o mesmo fotoperíodo. A combinação de temperaturas alternadas de 20 e 30 ºC com solução de 200 mg L^{-1} de GA_3 resultou em um aumento na porcentagem germinação de 19,2 % para 56,2 %, e do índice de velocidade de germinação de 0,19 para 3,12 com a consequente uniformidade na obtenção de plântulas normais.*

Palavras-chave: Pirênios, curva de embebição, tarumã, termoperíodo.

1 INTRODUCTION

Literature has demonstrated that endogenous compounds that promote and inhibit growth are directly involved in the germination process (ARAGÃO et al., 2003; PASSOS et al., 2004; SCALON et al., 2004). These substances may act alone or in combination with others, not only during the germination process but also in post-germination events such as energy reserve mobilization. Studies on the effect of gibberellic acid on seed germination of nonfruit woody species are limited to *Guarea guidonea* (CASTRO et al., 1999), *Cassia excelsa* (JÉLLER; PEREZ, 1999), *Jacaranda cuspidifolia* (SCALON et al., 2006), *Senna spectabilis* (JÉLLER; PEREZ, 2001), and *Talisia esculenta* (VIEIRA; GUSMÃO, 2006b).

Seed germination may demand gibberellins for one of the following stages: activation of the embryo's vegetative growth, weakening of the endosperm layer that involves the embryo and obstructs its growth, and mobilization of the energy reserves of the endosperm (TAIZ; ZIGER, 2008). According to these authors, applying gibberellins (GA_3) also stimulates the production of various hydrolases, including α-amylase, by aleurone layer cells of germinating cereal grains. During germination and initial growth of seedlings, endosperm reserves, particularly starch and protein, are hydrolized by various hydrolytic enzymes, and the solubilized sugars, aminoacids and other products are transported to the developing embryo. Gibberellin can be a limiting factor to α-amylase production during seed germination (CASTRO et al., 2004).

Gibberellin induces seed germination and promotes hypocotyl and stem elongation (PENG; HARBERD, 2000; RICHARDS et al., 2001). The use of growth regulators

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in seed germination is not as well disseminated with forest species as it is with fruit-bearing, vegetable, and medicinal species. While working with seeds of *Passiflora alata*, Rosseto et al. (2000) observed that using GA_3 at a concentration of 300 mg L^{-1} was more effective in promoting germination. Still with *Passiflora alata*, Ferreira (1998) observed that GA_3 at 100 mg L^{-1} promoted better germination, while with *Passiflora nitida* Passos et al. (2004) reported that for in vitro germination the most suitable gibberellic acid concentration was 1000 mg L^{-1}, with or without luminosity. Botelho and Perez (2001) reported significant effects on the germination percentage and speed with seeds of *Peltophorum dubium* Spreng (Taubert). With seeds of *Jatropha elliptica*, Añez et al. (2006) found higher germination percentages in treatments using gibberellic acid at concentrations of 300 to 500 ppm. In contrast, Vieira and Gusmão (2006a) argued that GA_3 failed to stimulate germination in a study with seeds of *Genipa americana*.

Studying *Caryocar brasiliense*, Souza and Salviano (2002) recommended using 1000 mg of GA_3 to two liters of water in order to increase germination and improve seedling growth. With the same species, Pereira et al. (2004) reported that, from 250 ppm of GA_3 onward, seed germination increased significantly. Similarly, Fuentes Fiallo et al. (1996) reported an increase in germination of oregano seeds (*Ocimum gratissimum*) with exogenous application of gibberellic acid starting at 250 ppm and as recommended for economic reasons, whereas applying the same substance for germination of the same species partially reverted light requirements (FACTOR et al., 2008).

In germination, response to temperature is dependent on species, variety, originating location and storage period. Some species require alternate temperatures to germinate while others are indifferent to that stimulus (CETNARSKI FILHO; NOGUEIRA, 2005).

Vitex montevidensis occurs in Atlantic Forest environment, from Bahia down to Rio Grande do Sul, and also in *Florestas de Pinhais*, *Cerradão* and *Florestas Semideciduas* of Paraná River basin. Fruits are consumed fresh or used for making candies and liqueur. The species can be used in conservation-oriented reforestation practices, providing both fruits to feed the local fauna and good quality wood (CARDOSO, 2004). However, the germination rate of *tarumã* seeds is less than 10% (LORENZI, 2000) and seed emergence is difficult where no-till planting is involved (CARRASCO et al., 2007).

The objective of this work is to evaluate the effects of GA_3 application and temperature on the germination of *tarumã* seeds.

2 MATERIAL AND METHODS

Reproductive structures of *tarumã* were collected in March 2005 from adult plants located in the municipality of Vera Cruz do Oeste (24°43'S and 53°48'W). Pyrenes were manually extracted using a surgical knife and choosing ripe fruits whose epicarp was color 1N according to Munsell color chart (MUNSELL, 1976). Pyrenes were subjected to treatment immediately after being extracted.

Variables being measured included germination percentage in sterilized sand arranged in plastic trays (40 x 25 x 8 cm) and germination speed index (MAGUIRE, 1962), in four replicates of 25 pyrenes each.

The quantification of gibberellic acid (GA_3) and temperature effects on germination used a completely randomized design in a 4x2 factorial consisting of four GA_3 concentrations and two thermoperiods. GA_3 concentrations being tested included zero, 50 mg L^{-1}, 100 mg L^{-1} or 200 mg L^{-1}, applied to pyrenes via immersion for 47 hours, according to preliminary data. Thermoperiods using BOD chambers (Fanen®, 347G model) consisted of alternate temperatures of 20ºC and 30ºC and constant 30ºC, both with 8 hours of light being provided by daylight fluorescent lamps (4x20 W) according to procedures suggested by Brasil (1992).

Different GA_3 concentrations were applied to the solutions containing pyrenes in 500 mL of distilled water, in beaker glassware with a capacity of 1 L, which were subjected to artificial air circulation using an aquarium pump (1.5 L min^{-1} pressure, 0.01Mpa) and water volume maintenance so as to prevent oxygen shortage in the solutions.

For the purpose of statistical analysis, data on germination percentage were transformed into arcsin $\sqrt{x/100}$ and germination speed index into $x + 0.5$ (SANTANA; RANAL, 2004). Results were subjected to analysis of variance and, when a statistical difference was found, means were compared using the Tukey test at the 5% probability level.

3 RESULTS AND DISCUSSION

Analyses of variance of germination percentage and germination speed index (IVG) data resulted in significant interaction ($P<0.05$) for the effects of applying GA_3 and temperature on the germination process (Table 1).

Table 1 – Germination percentage and germination speed index as a function of thermoperiod and GA_3 concentration.

Tabela 1 – *Germinação e índice de velocidade de germinação em função do termoperíodo e da concentração de* GA_3.

Thermoperiod		Concentration			
		0 mg L^{-1}	5 mg L^{-1}	100 mg L^{-1}	200 mg L^{-1}
Germination (%)	20-30 °C	19.2 Ca*	39.2 Ba	43.3 Ba	56.2 Aa
	30 °C	16.2 Ca	25.0 Bb	46.1 Aa	51.4 Ab
Germination Speed Index (IVG)	20-30 °C	0.19 Ca	1.05 Ba	1.62 Ba	3.12 Aa
	30 °C	0.18 Ba	0.59 Ba	2.16 Aa	2.44 Ab

*Means followed by the same lowercase letter in a column and uppercase letter in a line do not differ by the Tukey test (α = 0.05).

Results of growth regulator and temperature effects indicate that the use of GA_3 promoted an average germination increase in *tarumã* seeds from 17.7% (control) to more than 50% in treatments using 200 mg L^{-1} with any of the thermoperiods. The combination of alternate temperatures of 20ºC and 30ºC and a 200 mg L^{-1} GA_3 solution resulted in the highest germination percentage. When using a constant 30ºC thermoperiod, higher germination values were observed for GA_3 concentrations of 100 mg L^{-1} and 200 mg L^{-1}, which did not differ statistically.

Typically, initial species in secondary succession require temperature alternation in order to germinate (BRANCALION et al., 2010). The experimental results corroborate results reported by Santos and Aguiar (2005) with seeds of tropical shrub *branquilho* (*Sebastiania commersoniana* Bailon) using alternate temperatures of 20ºC and 30ºC, and also results found by Lopes et al. (2002) with seeds of *calabura* (*Muntingia calabura L.*), and Medeiros Filho et al. (2002) with seeds of *Operculina macrocarpa* (L.) Farwel and *Operculina alata* (Ham.) Urban.

In a study with seeds of *tarumã-branco* (*Citharexylum myrianthum* Cham.), Zanon et al. (1997) reported that seed germination values at 20ºC were statistically lower than germination values found at a constant temperature of 25ºC or 30ºC. However, these authors also reported that a temperature of 30ºC caused damage to seedling cotyledons and hypocotyls when vermiculite substrate was being used.

A higher germination speed index (IVG) was obtained from observations resulting from the combination of alternate 20ºC and 30ºC temperatures with 200 mg L^{-1} of GA_3, which did not significantly differ ($P<0.05$) from the combination between constant 30ºC temperature and 100 mg L^{-1} of GA_3. Results found for the germination speed index are similar to results found for germination percentage. These results are similar to those found by Agustin and Alviter (1996) with cherimoya seeds (*Annona cherimola* Mill.), who reported fast seed germination using gibberellic acid solutions of 150 mg L^{-1} to 200 mg L^{-1} for a period of twelve hours. Similarly, Ferreira et al. (2002) observed with seeds of sugar apple (*Anona squamosa* L.) that the highest concentration being tested (200 mg L^{-1}) provided the best germination results. Still on the subject of gibberellic acid for seed germination, other authors obtained better germination results under conditions similar to those described in this work (AÑEZ et al., 2006; FERREIRA, 1996; FUENTES FIALLO et al., 1996; GARCIA et al., 2006; ROSSETO et al., 2000).

Experimental results lead to the conclusion that improved germination percentage and germination speed index can be obtained with *tarumã* seeds if using alternate temperatures of 20ºC and 30ºC with 200 mg L^{-1} of GA_3 or a constant temperature of 30ºC with 100 mg L^{-1} or 200 mg L^{-1} of GA_3.

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