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ECOLOGICAL AND REPRODUCTIVE ASPECTS OF Syngonanthus caulescens RUHLAND (ERIOCAULACEAE) IN SÃO FRANCISCO DE ASSIS, RIO GRANDE DO SUL STATE, BRAZIL
Andressa Palharini Machado, Mara Lisiane Tissot-Squalli, Agatha do Canto Shubeita, Maicon da Silva Schreiber & Juliana Fachinetto

IMPORTÂNCIA DA CONSERVAÇÃO IN SITU DE *Copaifera langsdorffii* DESF. EM REMANESCENTES DE CERRADO, COM BASE EM PARÂMETROS DE ESTRUTURA E DIVERSIDADE GENÉTICA
Renata Gabriela Villegas de Castro e Souza, Lia Maris Orth Ritter Antiqueira & Paulo Yoshio Kageyama

SINOPSE DO GÊNERO DICRANELLA (MÜLL. HAL.) SCHIMP. (DICRANELLACEAE, BRYOPHYTA) PARA O BRASIL COM LECTOTIPIFICAÇÕES E CITAÇÕES DE NOVAS OCORRÊNCIAS
Dimas Marchi do Carmo & Denilson Fernandes Peralta

BRIÓFITAS DO PARQUE ESTADUAL DO FORNO GRANDE, ESPÍRITO SANTO - MATA ATLÂNTICA, BRASIL
Allan Laid Alkimim Faria, Daiane Valente Valente, Amanda Leal da Silva, Marcos João da Cunha, Eduardo Toledo de Amorim & Denilson Fernandes Peralta

BRIOFLORA ASSOCIADA A ARROIO RURAL NO MUNICÍPIO DE MORRO REDONDO, RIO GRANDE DO SUL, BRASIL, COM NOVAS OCORRÊNCIAS PARA O PAMPA
Elisa Teixeira Aires, Marinês Garcia & Juçara Bordin

BRIÓFITAS DE UM FRAGMENTO DE FLORESTA OMBRÓFILA DENSA, FAZENDA PATIOBA, ALAGOINHAS, BAHIA, BRASIL
Milena Evangelista & Gracineide Selma Santos de Almeida

FIRST REPORT OF *Entocybe haastii* (ENTOLOMATACEAE, AGARICOMYCETES) FROM BRAZIL
Fernando Augusto Bertazzo da Silva, Lilian Pedroso Maggio & Jair Putzke

OBSERVAÇÃO DE PLANTAS NA NATUREZA - UMA NOVA OPORTUNIDADE DE TURISMO ECOLÓGICO
Francielle Paulina de Araújo, Pamela Boelter Herrmann, Juçara Bordin & Felipe Gonzatti

PARÁBOLA FITOANTRÓPICA DAS MUDANÇAS TAXONÔMICAS
Josafá Carlos de Siqueira SJ.

COMPOSIÇÃO DA COMUNIDADE LIQUÊNICA NA ÁREA DA NASCENTE DO RIO DOS SINOS, CARAÁ, RS, BRASIL
Márcia Isabel Käffer, Vanessa Piasa, Daniela Dalke Weber, Jessica Fonseca de Araújo & Suzana Maria de Azevedo Martins

FITOPLÂNCTON DO PARQUE AQUÍCOLA PONTE PENSA, RESERVATÓRIO DE ILHA SOLTEIRA, SP
Edna Ferreira Rosini & Andréa Tucci

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Vasconcellea quercifolia A.St.-Hil. (CARICACEAE) GERMINATION UNDER GIBBERELLIC ACID INFLUENCE

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ABSTRACT

Vasconcellea quercifolia A.St.-Hil. (Caricaceae) is a native tree species to Brazil, with fast growth. It is included in the Unconventional Food Plants List, because of its potential for development of new food with functional properties. The aim of this study was to investigate gibberellic acid (GA3) influence on *V. quercifolia* germination. The experiment consisted of six treatments, as follows: control (without immersion in water) and immersion for four hours in solutions containing 0.0; 0.01; 0.03; 0.06 and 0.09 g L^{-1} GA3 concentrations. For each treatment, four repetitions of 50 seeds with a completely randomized design were used. Through germination monitoring, the following parameters were defined: germination percentage (PG), mean germination speed rate (IVGM), mean germination time (TMG), formed seedling percentage (PPC), mean seedling formation time (TMP) and seedling percentage in relation to the number of germinated seeds (RPFSG). Data were submitted to variance analysis (ANOVA), followed by polynomial regression. There was a positive effect for water immersion in relation to control, and IVGM, TMG, PPC and RPFSG variables obtained higher means. The highest PG and

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IVGM values were obtained in the treatments with the highest GA3 concentrations and linear growth was observed. In the 0.9 g L^{-1} concentration, PG and IVGM achieved the highest means, while the TMG and TMP values were lower. It can be concluded that gibberellic acid has a positive effect on the germination of *V. quercifolia*.
Keywords: Germination percentage; Native species; Seeds; Unconventional Food Plant.

RESUMO

Germinação de *Vasconcellea quercifolia* A.St.-Hil. (Caricaceae) sobre a influência do ácido giberélico*. Vasconcellea quercifolia* A.St.-Hil. (Caricaceae) é uma espécie arbórea nativa do Brasil, de rápido crescimento. Está incluída na lista das Plantas Alimentícias Não Convencionais, por apresentar potencial para o desenvolvimento de novos alimentos com propriedades funcionais. O objetivo do estudo foi verificar a influência do ácido giberélico (GA3) na germinação de *V. quercifolia*. O experimento foi composto pelos tratamentos: sem imersão em água e imersão por quatro horas em solução contendo 0,0; 0,01; 0,03; 0,06 e 0,09 g L^{-1} de GA3, cada um com quatro repetições de 50 sementes com delineamento experimental inteiramente casualizado. Foram definidos: percentual (PG) e índice de velocidade de germinação médio (IVGM), tempo médio de germinação (TMG), percentual de plântulas formadas (PPC), tempo médio de formação das plântulas (TMP) e percentual de plântulas em relação ao número de sementes germinadas (RPFSG). Os dados foram submetidos à análise de variância (ANOVA) seguido de regressão polinomial. Houve efeito positivo dos tratamentos com imersão em água com diferentes concentrações de GA3 em relação ao controle em todos os parâmetros avaliados. Os maiores valores de PG e IVG foram obtidos nos tratamentos com as maiores concentrações de GA3, observando-se um crescimento linear. Na concentração 0,9 g L^{-1}, o PG e IVGM alcançaram as maiores médias, enquanto os valores de TMG e TMP foram menores. Conclui-se que a imersão em ácido giberélico tem efeito positivo na germinação de *V. quercifolia.*
Palavras-chave: Porcentagem germinativa; Espécie nativa; Sementes; Planta Alimentícia Não Convencional.

INTRODUCTION

Brazil has one of the largest biodiversity on the planet (Pimentel *et al.,* 2015), it has high potential for the development of new food and pharmaceutical products. Therefore, species potential need to be investigated in order to assure that commercial exploitation does not exhaust natural resources that, on the opposite, it leads to ecological prudence and to social relevance (Barreiro; Bolzani, 2009; Alho, 2010).

Vasconcellea quercifolia A.St.-Hil. is a tree species popularly known in Brazil as “mamãozinho-do-mato” (oak-leaved papaya) or “jaracatiá”, occurring in the states of Goiás, São Paulo, Rio de Janeiro, Minas Gerais, Santa Catarina and Rio Grande do Sul (Santos, 1970), and it’s among the species with most potential for sustainable exploitation. The species is on the list of Unconventional Food Plants (UFP) (Kinupp; Lorenzi, 2014), since their fruits can be eaten *in natura* or as ice creams, jams and candies, for the last one the stem (medullary parenchyma) can be also used (Kinupp *et al*., 2011). The same authors mention that the latex of *V. quercifolia* has the papain enzyme, which has known use in both food and pharmaceutical industries (Kinupp *et al*., 2011). The aqueous fraction of this latex was described by Silva *et al.* (2019) as having antitumoral potential against squamous neoplastic cells of the human uterine cervix (SiHa), breast adenocarcinoma

(MCF-7) and non-tumour immortalized human keratinocytes (HaCat), with IC50 values after 24h in different concentrations for each cell lineage.

According to Siar *et al*. (2011), the species is source of proteases with the highest proteolytic activity in the genus. Also, Folharini *et al.* (2019) reported that the fruit, either ripe or green, as well as the medullary parenchyma, has high nutritional content, with high concentration of carbohydrates, proteins, carotenoids and ashes, with fibers being the component with higher content, reinforcing the species potential for developing functional food items.

Besides these properties, the species has a gene that makes it resistant to the *Papaya ringspot virus* (PRSV-P), which causes ring spots in papaya plants (*Carica papaya* L.) and results in big losses of productivity (Purcifull *et al*., 1984; Tripathi *et al.,* 2008). Thus, an intergeneric hybrid between *V. quercifolia* and *C. papaya*, developed by Siar *et al*. (2011), shows resistance to the above-mentioned virus. The species is also recommended to recover degraded areas and to conservation purposes (Kinupp *et al*., 2011).

Because of the many uses of the species, it should be among the main agricultural plants in the country with the possibility of being cultivated in agroecological systems, especially in family farming (Kinupp *et al*. 2011). Propagation by seeds is the most used method for seedling production, mainly because of the initial knowledge of native species, however the emergence occurs with desuniformity, and present poor quality due to genetic variability (Dias *et al.,* 2012). This can be corrected by using growth regulators as synthetic gibberellins, which speed the time, uniform the germination (Kerbauy, 2008).

The only study about *V. quercifolia* is the one by Pissatto (2015), in which studying the species it was verified 77,3% germination on vermiculite and the immersion in GA3 benefited the germination speed and percentage when the seed weren't stored. This same author emphasizes the importance of doing germination essays, because the species is not present in the list of Regras para Análise de Sementes (RAS) (Brasil, 2009), therefore demanding more studies about it.

Considering the species importance and the need to enable its sustainable exploitation for generating new products, obtaining papain or maintaining a germplasm bank, it is essential that studies about its germination are made. Thus, the present study had as its main objective to verify the influence of concentrations of gibberellic acid (GA3) on *V. quercifolia* germination.

MATERIAL AND METHODS

Collection site and bioassay conduction

The study was conducted between February and May 2015 in the Laboratório de Botânica of the Universidade do Vale do Taquari - Univates. Seeds were obtained from ripe fruits collected from seven specimens from different cities in the central region of Rio Grande do Sul state, Brazil, in a single day in February.

Seed cleaning and disinfestation

Seeds were removed from the fruits and manually washed with tap water with the aid of a sieve, in order to totally remove the pulp and mucilage. Subsequently, seeds were dried on paper towels. The desinfestation of the seed was performed on the bench. The seeds were kept in sodium hypochlorite solution (2.0-2.5% w/w) for 20 min, and then triple washed in distilled and autoclaved water.

Experiment Conditions

Plastic trays with 305 x 205 x 60 mm (length, width and height, respectively) dimensions that were disinfected with 96 percent alcohol were used for sowing. As substrate, 720 g dry sand hydrated with 80 mL distilled and autoclaved water were used. For experiment establishment, seeds were immersed during four hours in solutions containing different GA_3 concentrations, as follows: 0.1 g L^{-1} (TGA0.1), 0.3 g L^{-1} (TGA0.3), 0.6 g L^{-1} (TGA0.6) and 0.9 g L^{-1} (TGA0.9), besides immersion for four hours in distilled and autoclaved water (TH_2O) and no immersion (CT), totalizing six treatments, each with four repetitions of 50 seeds in a completely randomized design. These concentrations were selected were based in studies with other species, because of the lack of studies with *V. quercifolia*. After sowing, trays were covered with plastic wrap and kept in an environment with 16-h photoperiod, 466 Lux light intensity (fluorescent lamp) and 25 °C temperature (± 2 °C). Germination monitoring was carried out every three days, for 53 days, and the 2.0 mm radicle protrusion was used as a germination criterion. In order to define when the plant was formed, the emission of the first pair of leaves after leaf cotyledons was defined as a criterion. Substrate irrigation was performed with distilled water in the same days of germinated seeds evaluation using manual spray and the amount was defined by substrate visual inspection.

The procedures were based on studies with other species because of the lack of studies with *V. quercifolia.*

Assessed variables and statistical analyzes

For each treatment, germination percentage (PG), mean germination time (TMG), formed seedling percentage (PPC), mean seedling formation time (TMP), mean germination speed rate (IVGM) (Brasil, 2009) and seedling percentage in relation to the number of germinated seeds (RPFSG) (Maguire, 1962) were defined. Data were submitted to variance analysis (ANOVA) and afterwards the means were submitted to linear regression, using the CoStat software.

RESULTS

Germination assessment

Radicle emission occurred from the fifth day after sowing, continuing for up to 53 days. Water immersion treatment showed better results compared to the control treatment for PG, IVGM, TMG, PPC and RPFSG variables. PG and IVGM variable values were higher than the control mean as gibberellic acid concentrations increased, having linear growth pattern (Figures 1A and 1B).

Means obtained for PG and IVGM in the treatment with 0.9 g L^{-1} immersion (76% and 0.11, respectively) were higher than the other treatments, with $p < 0.01$ for PG, TMG, TMP, IVGM and with $p < 0.05$ for PPC and RPFSG variables for all concentrations used (Table 1).

Table 1. Mean values, standard deviation, P-value and coefficient of variation (%) found for *Vasconcellea quercifolia* in control, distilled and autoclaved water immersion and presoaking in gibberellic acid aqueous solution in concentrations of 0.1 g L-1, 0.3 g L-1, 0.6 gL-1 and 0.9 g L-1 for germination percentage (PG), mean germination speed rate (IVGM), mean germination time (TMG), formed seedling percentage (PPC), mean seedling formation time (TMP) e and seedling percentage in relation to the number of germinated seeds (RPFSG). *Significant at 5%. **Significant at 1%.

	PG	PPC	RPFSG	TMG	TMP	IVGM
Means	64.42	28.83	44.3	15.84	18.34	0.0778
Standard deviation	10.04	10.24	13.13	3.79	2.77	0.0198
p-value	0.002**	0.0197*	0.0302*	<0.001**	<0.001**	<0.001**
Coefficient of variation (%)	12.07	30.32	24.85	6.79	7.63	8.45

Formed seedlings assessment

For TMP (Figure 2), linear decrease mean seedling formation time was found. TG0.9 treatment had a mean of 15 days, with a significant difference in relation to the control, with 22 days. Formed seedling percentage (PPC) achieved higher results in treatments with GA3 in relation to control and distilled and autoclaved water immersion. The highest mean (37%) was obtained in the treatment with 0.1 g L^{-1}, followed by 0.9 g L^{-1} treatment, with 35%. For seedling percentage in relation to the number of germinated seeds (RPFSG), the 0.1g L^{-1} treatment obtained the best results (55%), followed by water immersion treatment, in which a 54% seedling rate was found.

DISCUSSION

The percentage of germination presented a linear grow with the increase of the concentration used, the highest value was obtained by the treatment 0.9 g L^{-1} differing from results obtained by Gerber *et al*. (2014), whose study was also carried out with *Vasconcellea quercifolia* using *in vitro* assays. Germination percentages were below 10% in the highest GA3 concentrations (0.1 g L^{-1} and 0.2 g L^{-1}) which, according to the authors, may have been a result of low GA3 concentrations.

In order to reinforce GA3 positive effects, a study conducted by Tokuhisa *et al.* (2007) showed better results for *Carica papaya* germination in both GA3 imbibition for 24h and GA3 application in the substrate (paper towel), in the concentration 0.6 g L^{-1}, in seeds without mucilage. Lopes *et al*. (2009), while assessing interaction between two maturity stages (50% and 75% of yellow surface) and GA3 action in *C. papaya* germination, observed that there was no germination in the control treatment (without GA3 use) compared to treatments with GA3, regardless of maturity stage. In the present work there were tested mature fruits and seeds without mucilage, factors that may have influenced germination, considering that studies cited previously tested these factors together with the GA3 concentrations with a species of the same family of *V. quercifolia.* Likewise, Batista *et al*. (2012), studying *Handroanthus impetiginosa* (Mart. ex DC.) Mattos, found increasing germination percentage values through imbibition of the seeds with GA3 aqueous solution in concentrations of 0.04 and 0.08 g L^{-1} in the 60 minutes period (60.25% and 63.25%, respectively). Still another study showing GA3 positive effect was

conducted by Kissmann *et al*. (2011) with *Jacaranda decurrens* subsp. *symmetrifoliolata* Farias & Proença germination was higher than other treatment when of seeds were immersed in the concentrations with 0.1 and 0.2 g L^{-1} GA3.

Oliveira *et al*. (2013), while studying *Acrocomia aculeata* (Jacq.) Lodd. ex. Mart., found that there was higher IVG in the 2 and 5 g L^{-1} GA3 concentration, significantly differing from control, and the immersion in 48 hours period provided the highest result. Similar results were obtained in this study, showing an increasing trend that was proportional to gibberellic acid concentration increase. Guimarães *et al.* (2010), in studies with *Thlaspi caerulescens* J. Presl & C. Presl germination, found higher GSI (germination speed index) results in seeds soaked with GA3 at temperatures of 15 to 20 °C, both with light presence or absence. Neto *et al*. (2015) studied arboreal tomato (*Solanum betaceum* Cav.) germination and found higher IVG values with 100 and 300 ppm (0.1 and 0.3 g L^{-1}) GA3 concentrations, both in the presence and absence of light. According to Hooley (1994), GA3 facilitates seed germination, as it stimulates embryo growth and promotes the production of hydrolases that weaken the endosperm structure surrounding the embryo.

Regarding the mean germination time in the present study, seeds germinated in a lower mean time in treatment with GA3 immersion. Similar results were found by Neto *et al*. (2015) while studying arboreal tomato germination, with positive results in 100 and 300 ppm GA3 concentrations, decreasing by 13.5 and 16.87 days in relation to the control treatment, with a mean time of 37.58 days. In a *Caryocar brasiliense* Camb. study, Dombroski *et al*. (2010) found that the TMG was lower in the treatments in which the paper was soaked in GA3 at the concentration of 1 µmol L^{-1} GA3 (approximately 0.0796 g L^{-1}). Seedling mean formation time was lower in treatments with gibberellic acid. Bernardes *et al.* (2008) reported that gibberellic acid accelerates germination and reduces mean germination time of *Caryocar brasiliense* Camb., and it uniformed the germination.

CONCLUSIONS

Vasconcellea quercifolia seeds immersed in different GA3 concentrations showed higher results for all the studied variables, showing that gibberellic acid accelerates germination, favoring seedling production.

Further studies should be performed considering other factors such as the stage of maturation and the presence of mucilage.

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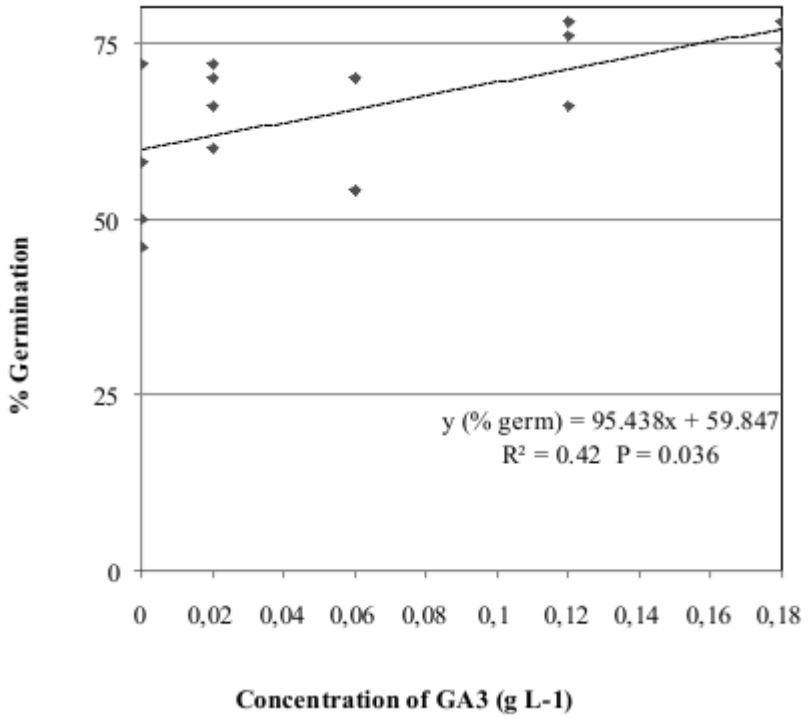
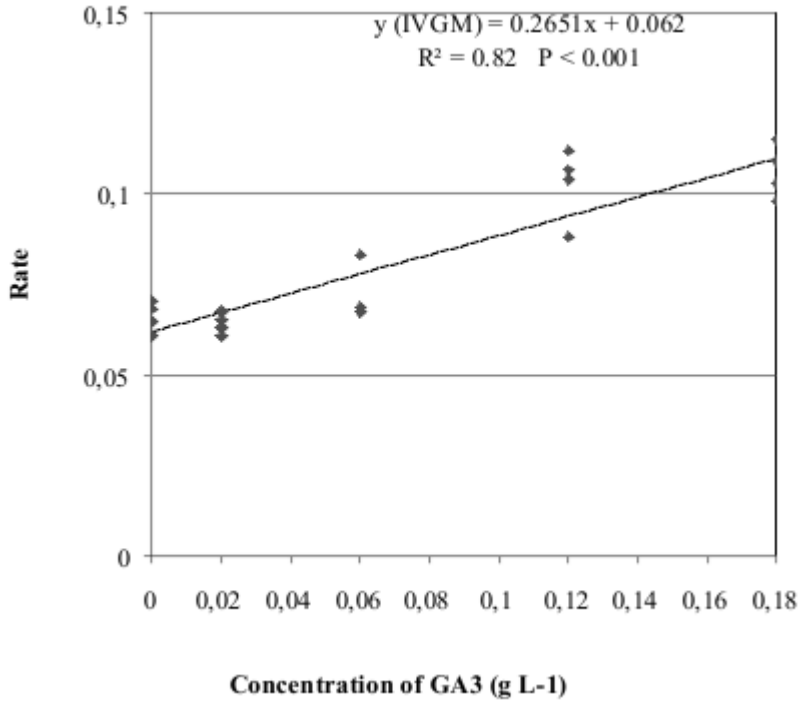


[A]



[B]

Figure 1. *Vasconcellea quercifolia g*ermination in seeds treated with gibberellic acid (GA_3) for germination percentage (PG) (A) and mean germination speed rate (IVGM) (B).

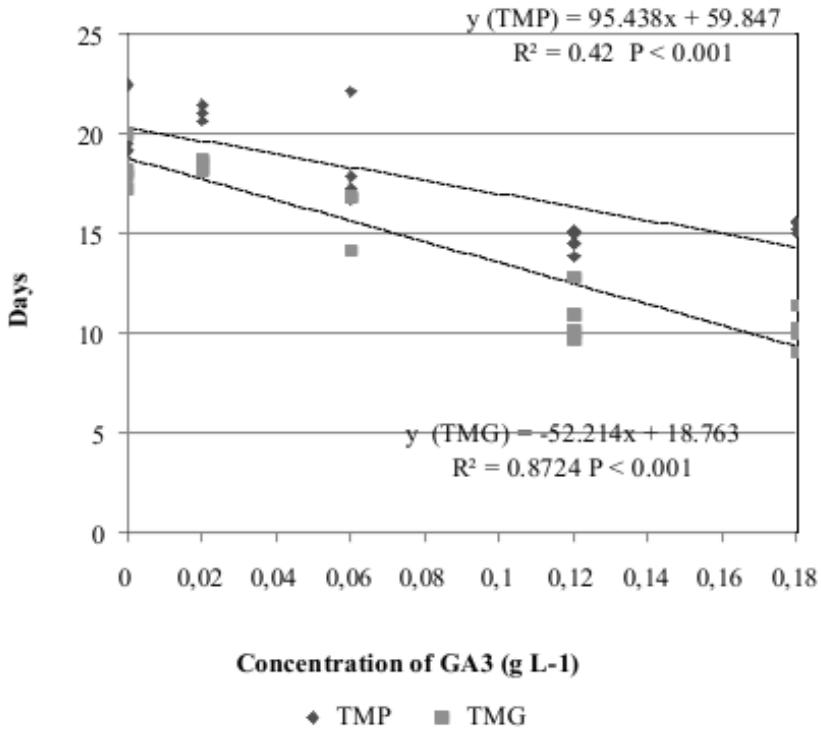


Figure 2. *Vasconcellea quercifolia* germination in seeds treated with gibberellic acid (GA3) mean germination time (TMG) and plant formation (TMP).