

Germination of *Mansonia altissima* (A. Chev.) A. Chev. var. *altissima*: an endangered valuable timber species in Africa

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ABSTRACT. The *in vitro* seed germination which results in the production of disease-free seedlings and greenhouse germination of the seeds of *Mansonia altissima* was investigated in order to establish a better way of germination of the timber species. Five levels of GA₃ treatment were used in *in vitro* germination with three replicate and two seeds were inoculated in each of the jam bottle. Whereas, in greenhouse germination, five levels of different treatments were used, replicated three times and each Petri plate contained 15 seeds. The experiment was repeated twice and the data from each experiment was put together and used for the statistical analysis. The results showed that seeds germination occurred eight days after inoculation in *in vitro* but in the case of greenhouse germination, it took only five days. For *in vitro* rapid germination of *Mansonia altissima*, the MS medium should be supplemented with 1.0 µm of GA₃. Equally, in greenhouse germination, the seeds need to be soaked in 1.0 mM of GA₃ for 24 hours. Alternatively, in the absence of GA₃, the seeds can be soaked in water for 24 hours before broadcasting the seeds on the seedbed for germination, as this will help to identify nonviable seeds.

Keywords: MS médium; GA₃; *in vitro*; growth parameters; greenhouse; seed.

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Introduction

Mansonia altissima (A. Chev.) A. Chev. var. *altissima* is a tree species which belongs to the family of Malvaceae. The tree species can be found in the Republic of Benin, the Democratic Republic of Congo, Cote d'Ivoire, Ghana, Guinea, Nigeria, Sudan, Togo, and Uganda where it is commonly called Mansonia, African black walnut, African walnut. *Mansonia altissima* is a valuable timber, commonly harvested from the wild and exported, especially to the USA and Europe, where it is being used as a substitute for walnut (Osunlaja, Olomola, Nwogwugwu, Afolabi, & Oloyede, 2017). The wood of *Mansonia altissima* is used for general and high-class joinery, cabinet work, furniture, turnery, decorative veneer, and handicrafts. It is also used in construction of doors and windows (Osunlaja et al., 2017). The products from the bark of *Mansonia altissima* have been used in the treatment of leprosy. Again, extracts from the bark have been shown to inhibit the growth of *Mycobacterium tuberculosis* (Fernandez-Villamil, Dubin, Galeffi, & Stoppani, 1990). A decoction of the twig bark is applied as a bath against yaws, scabies, and syphilis. Moreover, ethanolic extract of the wood has shown hepatotoxic and haematotoxic effects when administered orally. The bark contains a highly toxic compound known as mansonine, which is related to cardenolides from *Digitalis purpurea* and ouabain from *Strophanthus preussii* (Akinagbe, Gailing, & Finkeldey, 2010; Fernandez-Villamil et al., 1990).

International Union for Conservation of Nature's (IUCN, 1998) red list of threatened species included *Mansonia altissima* (A. Chev.) A. Chev. var. *altissima* as one of the endangered valuable timber species (IUCN, 1998). The timber becomes endangered due to overexploitation for furniture, cabinet, and demands from foreign countries and inadequate of the seeds for germination because of its recalcitrant and dormancy nature (Wédjangnon, Houèthégnon, & Ouinsavi, 2016). These situations trigger depletion of the timber species in forest as demand for the species for wood production could no longer cope with their supply. Consequently, the species is moving towards extinction, reduction in biodiversity and decrease in primary productivity. With the immense importance of this timber species, there is a need to improve the germination techniques of the plant *in vitro* and in the field.

Seed germination and rapid germination are usually essential processes in seedling establishment, especially with plants that are endangered (Murungu, Nyamugafata, Chiduzi, Clark, & Whalley, 2003). Many studies such as Maku, Gbadamosi, and Fadoju (2014) and Dentaa, Wiafe, and Nutsuakor (2015) evaluated the germination success only based on final percentage germination, however it is not enough due to the lack of ability to compare two sets of data (one lot of seed may have germinated well before the other, but both attained the same final germination percentage) and this has led to the development of a number of germination parameters (Jeyavanan, Sivachandiran, Vinujan, & Pushpakumara, 2016; Almaghrabi, 2012). In the case of *Mansonia altissima*, the majority of studies had focused only on the final germination percentage and this has been a setback in the species germination understanding. Hence, this study investigates suitable treatment for the seed germination by calculating the germination parameters of *Mansonia altissima* treated with different chemicals.

Material and methods

Study site

The experiment was carried out at Department of Biotechnology, Bhimtal Campus, Kumaun University Nainital, Uttarakhand, India. Bhimtal is situated at latitude 29° 35' N and longitude 79° 57' E.

Collection of seeds

Mansonia altissima seeds were collected from Forest Research Institute of Nigeria, Jericho Hill, Ibadan, Oyo State, Nigeria (Latitude 7° 30' N and Longitude 3° 54' E).

Seeds sterilization for *in vitro* germination

The seeds of *Mansonia altissima* were soaked in water (at room temperature) for 24 hours after which they were kept under running tap for 30 minutes for proper washing, and then washed in 2% of Tween 20 for 20 minutes. The seeds were then treated with 1% antifungal (Bavistin) for 15 minutes, washed with sterilized distilled water and transferred to the laminar flow. Under the laminar flow, the seeds were treated with 0.1% HgCl₂ for 1–2 minutes and 0.5% streptomycin for 5 minutes and lastly, 70% ethanol for 1 minute before being washed in sterilized distilled water for 5 times.

Seeds treatment for greenhouse germination

The seeds of *Mansonia altissima* were soaked in water, 1 mM of GA₃, 1 mM of GA₃ + 0.2% KNO₃ and 0.2% KNO₃ for 24 hours. Whereas, un-soaked seeds were used as control.

Experimental design

The Murashige and Skoog (1962) (MS) medium with the pH 5.8, was supplemented with five levels of gibberellic acid (GA₃) in the *in vitro* germination (0 (Basal medium), 0.1, 1.0, 3.0 and 5.0 µm) with three replicate and two seeds in each jam bottle. Whereas in Petri plate germination, five levels of treatment (un-soaked, soaked in water, soaked in 1 mM of GA₃, 1 mM of GA₃ + 0.2% KNO₃ and 0.2% KNO₃), was investigated, which were replicated three times and each Petri plate containing 15 seeds. The experiment was repeated twice, and the data obtained from each experiment was put together and used for the statistical analysis.

Germination condition

The inoculated seeds were kept in a growth room with temperature 28±2°C, average light intensity was 4300 lux, photoperiod 16 h⁻¹ hours and covered with black cloth. Also, the seeds in each of the Petri plates were covered with cotton wool because of the floating nature of the seeds and kept in greenhouse with temperature 25±2°C, relative humidity 60%, average light intensity was 22900 lux and 16 h⁻¹ hours photoperiod.

Germination parameters

The following germination parameters were considered (Gashi, Abdullahi, Mata, & Kongjika, 2012; Kader, 2005).

1. Final germination percentage (FGP):

$$FGP (\%) = \frac{\text{no of germinated seeds}}{\text{Total no of seeds}} \times 100$$

2. Mean germination time (MGT):

$$MGT (\text{day}) = \frac{\sum FX}{\sum F}$$

Where F is the number of seeds which germinated on day X, X is the number of days counted from the beginning of germination

3. First day of germination (FDG): The day on which the first germination occurred

4. Last day of germination (LDG): The day on which the last germination occurred

5. Coefficient of velocity of germination (CVG):

$$CVG (\%) = \frac{(N_1 + N_2 + N_3 + \dots + N_x)}{N_1 T_1 + N_2 T_2 + N_3 T_3 + \dots + N_x T_x} \times 100 \text{ or } \frac{\sum N_i}{\sum N_i T_i}$$

where N = number of seeds germinated each day, T = number of days from seeding corresponding to N

6. Germination rate index (GRI):

$$GRI (\%|\text{day}) = \frac{G\%_1}{1} + \frac{G\%_2}{2} + \frac{G\%_x}{x}$$

G%₁ = germination percentage at first day, G%₂ = germination percentage at second day G%_x

7. Corrected germination rate index (CGRI):

$$CGRI (\%|\text{day}) = \frac{(GRI)}{(FGP)} \times 100$$

8. Time spread of germination (TSG): The time in days between the first and last germination events occurring in a seed lot.

Analysis of data

Data obtained from each of the experiments conducted was put together and the data were subjected to one-way analysis of variance (ANOVA) using SAS 9.1 version software (Oseni, Adelusi, Dada, & Rufai, 2016; Oseni, Dada, Okunlola, & Ajao, 2018). The means were separated using fisher's LSD at the probability level of 0.05 ($p < 0.05$).

Results and discussion

Seed germination

Germination is the process that begins with the uptake of water by the dry seed and ends with the emergence of the embryonic axis, usually the radicle, from its surrounding tissues. Germination requires water, temperature, oxygen, light, and nitrate. Of all these, water is the most essential factor. Under normal conditions, water uptake by dry seed is triphasic. This begins with a rapid initial uptake (phase I, *i.e.* imbibition) because water flow from the higher to the lower water potential. Then, followed by declines in water uptake by imbibition and metabolic processes are reinitiated (phase II). A further increase in water uptake (phase III) occurs as the embryo axis elongates and breaks through the covering layers to complete germination (Taiz, Zeiger, Møller, & Murphy, 2015). Seed is considered as germinated upon radicle emergence ≥ 0.5 mm (Charu, Ravi, & Tapan, 2016).

After 24 hours of soaking, the seed did not sink as shown in Figure 1a. Viable seeds were determined by pressing each seed with the fingers. Seeds that were soft when pressed were considered not to be viable. Whereas, seeds that were still hard when pressed were considered viable. Also, Figure 1b showing the selected viable seeds inoculated on MS medium supplemented with GA₃ for germination.

As shown in Figure 2, *in vitro* seed germination occurred eight days after inoculation and lasted for 11 days. The total average percentage of *in vitro* seed germination was 96%. Germination started with MS medium supplemented with 1.0 μm of gibberellic acid (GA₃) and the germination lasted for 2 days. The

highest germination percentage for *in vitro* seed germination occurred in MS medium supplemented with 1.0 μM of GA_3 (75%) and occurred on the first day of germination. Followed was MS medium supplemented with 0.1 μM of GA_3 but showed the highest germination percentage on the third day of germination (70%) and the germination lasted for 4 days. Whereas MS medium supplemented with 3.0 μM and 5.0 μM of GA_3 showed germination on the fourth day after germination had started, but their germination lasted for five and six days respectively.

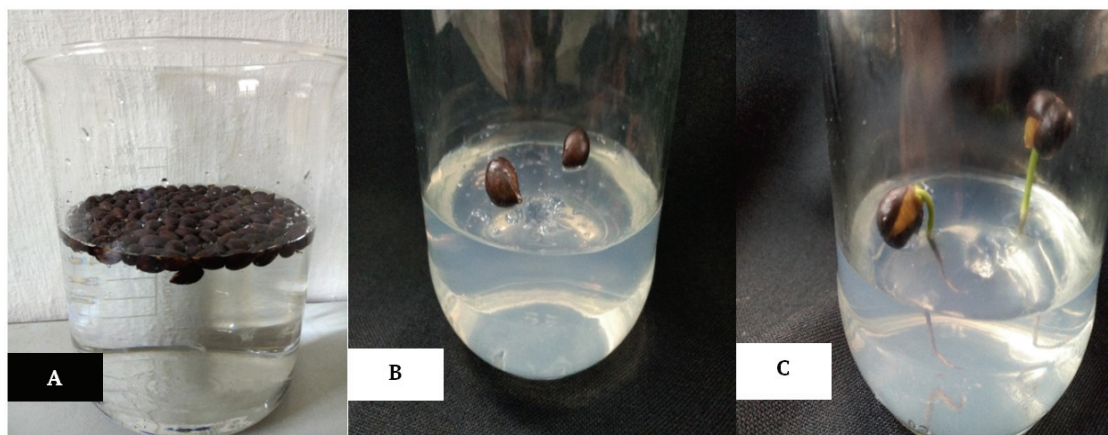


Figure 1. Germination of *Mansonia altissima*. (a): seeds soak for 24 hours (b): Inoculated seeds (c): germination of inoculated seeds.

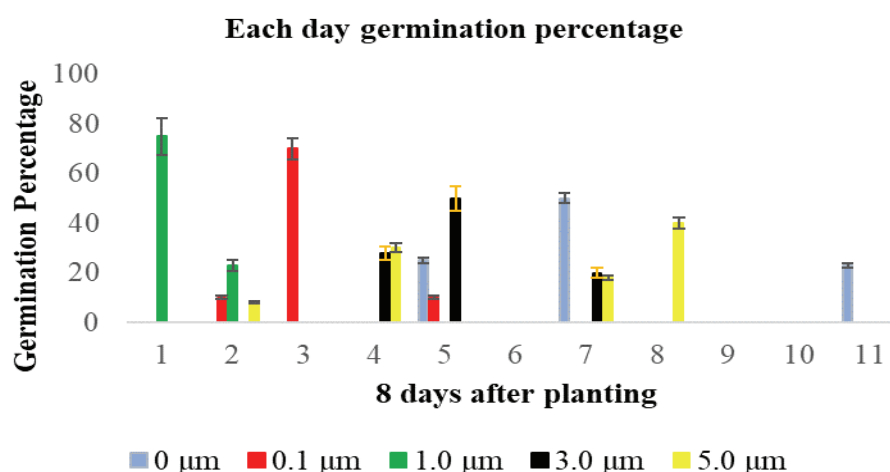


Figure 2. *In vitro* germination percentage per day of *Mansonia altissima*

In greenhouse germination, shown in Figure 3, the seed germination started on the fifth day after setting up the Petri plate and lasted for 16 days. Germination started with seeds soaked in 1 mM of GA_3 for 24 hours which had the highest percentage of germination (51%) on the second day of germination and the seed germination lasted for 4 days. seeds soaked in 1.0 mM of GA_3 + 0.2% KNO_3 for 24 hours had the highest germination percentage (54%) on the first day of germination and the germination lasted for 3 days. Seeds soak in 0.2% KNO_3 for 24 hours germinated on the fifth day after germination had started, with the highest germination percentage (60%) on the seventh day of germination. Moreover, seeds soaked in water for 24 hours germinated on the sixth day of germination and the germination lasted for 3 days, which had the highest germination percentage (54%) on the eighth day of germination. Seeds that were non-soaked germinated last which had the highest germination percentage (40%) on the eleventh day of germination and the germination lasted for 6 days.

Seed germination occurred eight days after inoculation in *in vitro* germination, whereas it took only five days in greenhouse germination, this might be as a result of adequate water supplied to seeds planted in Petri plate but the incubated seeds only got their water from the one-liter prepared MS medium. As water is the major requirement for seed germination (Taiz et al., 2015). Maku et al. (2014) also observed the same germination period (five days after planting) with *Mansonia altissima* when treated with different plant growth hormone. However, germination of *Mansonia altissima* started two weeks after sowing according to

the study of Dentaa et al. (2015), the reason may be because the seeds were sowed in the soil and germination might have occurred, or because the seeds were not treated with phytohormone.

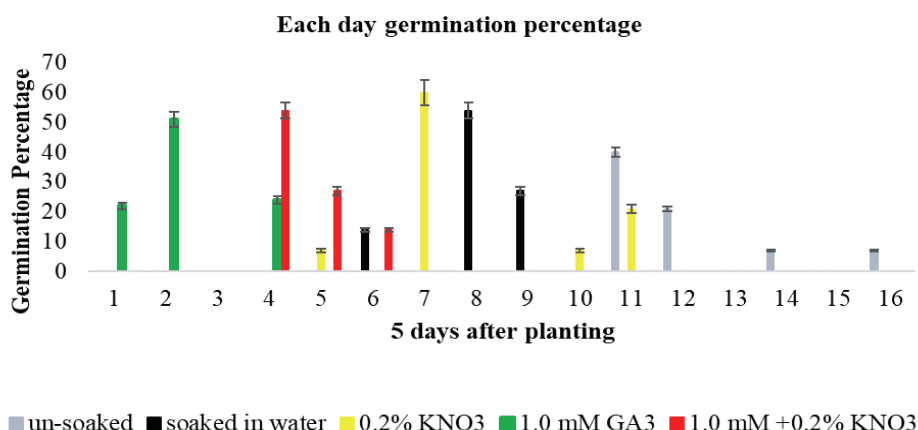


Figure 3. Greenhouse germination percentage per day of *Mansonia altissima*

Germination occurred firstly in seeds treated with GA₃ in both experiments although at lower level in *in vitro* germination. GA₃ had been reported to induce seeds germination better than other plant hormones and showed effects at very low concentration (Finch-Savage & Leubner- Metzger, 2006). The ratio of ABA and GA₃ is the primary determinant of seed dormancy. A higher level of ABA in seed imposes dormancy on seed whereas a higher level of GA₃ makes the seed to be nondormant. GA₃ stimulates seed germination via amylase synthesis (Finch-Savage & Leubner-Metzger, 2006; Gashi et al., 2012). According to Gashi et al. (2012), GA₃ and KNO₃ enhanced seed germination of *Ramonda serbica* and *Ramonda nathaliae*. The authors also supported the findings of Charu et al. (2016), which is also in line with the results gotten from Petri plate germination when soaked in 1.0 mM of GA₃ + 0.2% KNO₃ for 24 hours. The seed exhibit epigeal type of seed germination, as shown in Figure 1c.

Germination parameters

The germination parameters of *in vitro* germination were shown in Table 1. Seeds inoculated in MS medium supplemented with 1.0 µm, 3.0 µm of GA₃ and basal medium had the highest FGP (98%) which was significantly higher than others ($p < 0.05$). Basal medium had the highest MGT (6.7 days), and next to it was MS medium supplemented with 5 µm of GA₃ (6.1 days). The lowest MGT was 1.3 days, which was observed in MS medium supplemented with 1.0 µm of GA₃. MS medium supplemented with 1.0 µm of GA₃ had the lowest FDG (8 days) but not significantly different ($p < 0.05$) from MS medium supplemented with 0.1 µm and 5.0 µm of GA₃, whereas basal medium showed the highest FDG (12 days). Furthermore, MS medium supplemented with 1.0 µm of GA₃ showed the lowest LDG (9 days), and basal medium had the highest LDG (18 days). The highest CVG, GRI and CGRI were observed in MS medium supplemented with 1.0 µm of GA₃ followed was MS medium supplemented 0.1 µm of GA₃, and basal medium had the lowest. Basal medium and MS medium supplemented with 5.0 µm of GA₃ had the highest TSG (6 days), followed by MS medium supplemented with 0.1 µm and 3.0 µm of GA₃ (3 days).

As shown in Table 2, the germination parameters of *Mansonia altissima* germinated in the greenhouse. The FGP range from 75% to 97%, with un-soaked seeds having the lowest and seeds soaked in 1.0 mM of GA₃ had the highest. In addition, the highest MGT was observed in un-soaked seeds (12.1 days). Followed were seeds soaked in water and 0.2% KNO₃ (8.1 and 8 days respectively) but no significant difference ($p < 0.05$). The seeds soak in 1 mM of GA₃ had the lowest MGT (2.3 days), next to it was seed soaked in 1.0 mM of GA₃ and 0.2% KNO₃ (4.6 days). The FDG range from 5 to 15 days and the LDG range from 8 to 20 days, which occurred in seeds soaked in 1.0 mM of GA₃ and un-soaked seeds respectively. The CVG, GRI, CGRI were in order of seeds soak in 1.0 mM of GA₃ > 1.0 mM of GA₃ + 0.2% KNO₃ > 0.2% KNO₃ > seeds soaked in water > un-soaked. Seeds soaked in 0.2% KNO₃ had the highest TSG (6 days) and seeds soaked in 1.0 mM of GA₃ + 0.2% KNO₃ had the lowest TSG (2 days).

Germination parameter is considered to be a qualitative developmental response of an individual seed that occurs at a point in time within a treatment respond within different time (Gashi et al., 2012; Jeyavanan et al., 2016; Murungu, 2011; Rastegar, Sedghi, & Khomari, 2011; Kader, 2005). According to Jeyavanan et al.

(2016), the higher the FGP values, the greater the germination of a seed population. All the treatment in the experiment showed greater value of FGP, which means that majority of the seeds are viable. Except the un-soaked seeds, which might be as a result of not being able to identify the nonviable seeds through pressing by fingers. The lower the MGT, the faster a population of seeds has germinated (Rastegar et al., 2011). Lower FDG values indicate a faster initiation of germination (Kader, 2005). Lower LDG values indicate a faster ending of germination (Kader, 2005). Seeds inoculated in MS medium supplemented with 1.0 μM of GA_3 and seeds soaked in 1.0 mM of GA_3 in Petri plate germination showed the lowest number for MGT, FDG, and LDG. These results indicate that the seeds started and completed their germination within the shortest period which make the treatment more effective than others. As defined by the following authors: Darshini and Aruna (2014), Kader (2005), Shahriari, Puteh, Saleh, and Abdul-Rahim (2014), Sharafizad, Naderi, Siadat, Sakineja, and Lak, (2013), the CVG gives an indication of rapid germination, it increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest CVG is 100, and this occurs only if all the seeds germinated on the first day. The GRI reflects the percentage of germination on each day of the germination period. The higher the values of GRI and CGRI, the higher and faster the germination. The higher the TSG values, the greater the difference in germination speed between the 'fast' and 'slow' germinating members of a seed lot. Higher value of CVG, GRI and CGRI occurred in seeds inoculated in MS medium supplemented with 1.0 μM of GA_3 and seeds soaked in 1.0 mM of GA_3 in *in vitro* and Petri plate germination, which showed that, the two treatments were suitable for rapid germination of *Mansonia altissima*. Finally, the TSG values indicated that, there were differences in time of germination.

Table 1. Germination parameters of *Mansonia altissima* grown *in vitro*

Treatment	Germination parameter							
	FGP (%)	MGT (day)	FDG (day)	LDG (day)	CVG (%)	GRI (% day ⁻¹)	CGRI (% day ⁻¹)	TSG (day)
0 μM	98.0 ^a ±0.6	6.7 ^a ±1.2	12.0 ^a ±1.2	18.0 ^a ±0.6	13.5 ^e ±0.1	14.2 ^e ±1.6	14.5 ^e ±1.5	6.0 ^a ±0.6
0.1 μM	90.0 ^c ±0.5	3.1 ^d ±1.7	9.0 ^b ±2.3	12.0 ^d ±0.5	32.1 ^b ±0.1	30.3 ^b ±3.1	33.7 ^b ±4.7	3.0 ^b ±0.6
1.0 μM	98.0 ^a ±0.6	1.3 ^e ±0.6	8.0 ^b ±2.3	9.0 ^e ±0.6	81.0 ^a ±0.1	86.5 ^a ±2.7	88.3 ^a ±6.4	1.0 ^c ±0.3
3.0 μM	98.0 ^a ±0.4	5.1 ^c ±1.7	11.0 ^a ±1.7	14.0 ^c ±0.6	19.5 ^c ±0.1	19.9 ^c ±4.9	20.3 ^c ±2.8	3.0 ^b ±0.6
5.0 μM	96.0 ^b ±0.6	6.1 ^b ±1.2	9.0 ^b ±1.7	15.0 ^b ±0.5	16.5 ^d ±0.1	19.1 ^d ±2.3	19.9 ^d ±1.0	6.0 ^a ±0.5

FGP- final germination percentage, MGT- mean germination time, FDG- first day of germination, LDG- last day of germination, CVG- coefficient of velocity of germination, GRI- germination rate index, CGRI- corrected germination rate index, TSG- time speed of germination, MDG- mean of daily germination. Means with the same letters within the column are not significantly different at $p < 0.05$

Table 2. Germination parameters of *Mansonia altissima* germinated in greenhouse

Treatment	Germination parameter							
	FGP (%)	MGT (day)	FDG (day)	LDG (day)	CVG (%)	GRI (% day ⁻¹)	CGRI (% day ⁻¹)	TSG (day)
Un-soaked	75.0 ^c ±1.2	12.1 ^a ±1.4	15.0 ^a ±0.9	20.0 ^a ±0.6	8.3 ^d ±1.3	6.3 ^e ±0.9	8.4 ^e ±0.9	5.0 ^{ab} ±0.6
Soaked in water	95.0 ^b ±2.0	8.1 ^b ±0.9	10.0 ^b ±0.3	13.0 ^c ±0.9	12.5 ^c ±1.1	12.1 ^d ±1.6	12.7 ^d ±1.7	3.0 ^{bc} ±0.3
0.3% KNO ₃	95.0 ^b ±1.7	8.0 ^b ±1.4	9.0 ^{bc} ±0.6	15.0 ^b ±0.9	12.6 ^c ±1.2	12.6 ^c ±2.4	13.3 ^c ±1.7	6.0 ^a ±0.9
1.0 mM GA ₃	97.0 ^a ±0.3	2.3 ^d ±0.7	5.0 ^d ±0.7	8.0 ^e ±0.6	44.1 ^a ±3.3	53.5 ^a ±4.7	55.2 ^a ±2.4	3.0 ^{bc} ±0.6
1.0 mM +0.2% KNO ₃	95.0 ^b ±0.7	4.6 ^c ±1.0	8.0 ^e ±1.0	10.0 ^d ±0.9	21.8 ^b ±1.8	21.2 ^b ±1.4	22.3 ^b ±1.6	2.0 ^c ±0.6

FGP- final germination percentage, MGT- mean germination time, FDG- first day of germination, LDG- last day of germination, CVG- coefficient of velocity of germination, GRI- germination rate index, CGRI- corrected germination rate index, TSG- time speed of germination, MDG- mean of daily germination. Means with the same letters within the column are not significantly different at $p < 0.05$

Conclusion

The results of the experiment showed that, *in vitro* germination of *Mansonia altissima* seeds, required MS medium supplemented with 1.0 μM of GA_3 . Also, in greenhouse germination, soaking the seeds in 1.0 mM of GA_3 for 24 hours will also be perfect for seed germination. Alternatively, in the absence of GA_3 , the seeds can be soaked in water for 24 hours before broadcasting the seeds on the seedbed for germination, as this will help to identify nonviable seeds.

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