J. Bamboo Rattan (2023) 22(3): 57-64
https://doi.org/10.55899/09734449/jbr022302 www.jbronline.org
RESEARCH ARTICLE

Response of GA_3, NAA, and KNO_3 treatments on in vitro seed germination and shoot performance of highland bamboo (*Oldeania alpina L.*)

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Received: 12 June 2023/Accepted: 20 November 2023
Published online 21 October 2024

Abstract: *Oldeania alpina* (*K. Schum*) Stapleton is an African alpine bamboo, native of Ethiopia to Zambia. The untreated seeds of *O. alpina* do not give good germination. Therefore, the effects of different seed pretreatments on germination and shoot quality were evaluated. The presowing treatments were highly significant ($P \leq 0.001$) for all studied parameters. The seeds subjected to 8% Gibberellic acid (GA3) treatment produced the highest germination percent (30%) while 6% NAA naphthalene acetic acid produced 23% germination. The values of MGT (*mean germination time*), PV (*peak value*)and VI (*vigorous index*) were also analyzed and interpreted to evaluate the germination of seed and shoot quality of the emerging plants. Moreover, the highest values for morphological parameters were recorded for 8% GA3 and 6 % NAA. Hence, the use of 8% GA3 or 6% NAA pretreatment is recommended to enhance the germination and shoot quality of *O. alpina.*

Keywords: germination, growth regulators, nutrients, plantlets quality, pre-treatment

Introduction

Highland bamboo *(Oldeania alpina L)* in Ethiopia *is* mainly propagated vegetatively using different plant parts (Konzen *et al*., 2021; Paembonan *et al*., 2019; Kebede *et al*., 2017). Propagation from seed, however, is cost effective and essential to maintain the sustainability of genetic material, besides its cost-effectiveness. As a biological product, seed behavior and its germination also vary between species and seed lots, and can be influenced by the interaction of genetics and habitat (Bahru *et al.,* 2015; Fredrick *et al.,* 2015; Derero *et al.,* 2012; Loha *et al.,* 2006; Mamo *et al.,* 2006). Germination capacity and time to germinate seed are determined by seed weight and size, which are also strongly influenced by genotype and habitat (Carles *et al.,* 2009; Norden *et al.,* 2008).

Various seed pretreatments are required to overcome germination challenges to produce initial seedlings from stored seeds with low viability. Plant growth regulators and inorganic nutrients have been commonly used pretreatments. Studies have shown important roles for abscisic acid and gibberellic acid in regulating dormancy and improving germination ((Dev *et al*., 2020; Khodadadi *et al*., 2018; Jyoti *et al*., 2016; Maku *et al*., 2014; Cárdenas *et al*., 2013; Gashi *et al*., 2012; Kornneef *et al*., 2002). Gibberellic acid is one of the most commonly used growth regulators that promote germination (Tuan *et al*., 2018). Improving germination and shoot quality are well studied using plant growth regulators and nutrients, but little research has been done on *O. alpina* seed priming. Therefore, this study was initiated to evaluate the effect of plant growth regulators and inorganic nutrients on *O. alpina* germination and shoot development in order to promote nursery seedling production.

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Materials and methods

Study area

This study was conducted in the Tree Seed Quality Control Laboratory (latitude 9° 01′ 04″ N and longitude 38° 49′ 06″ E, which is 2377 masl elevation) of the Central Ethiopian Forestry Development Center in Addis Ababa. The sample *O. alpina* seeds were taken from a cold room (5 °C) where they were stored for 3 months after being processed and tested for seed quality following the standard methods (FAO, 1985). The seed had 88.4% purity, 5.4% moisture content, and had a seed length of 0.47 cm and width of 0.25 cm. The seed source material was collected from Desta site bamboo vegetation at Adeyo district in Kefa Zone, South-West Ethiopia (between 7° 8' to 7° 26'N latitude and 36o 15' to 36o 50'E longitude). The elevation ranges from 500 to 3000 m above sea level.

To determine the pretreatment effect on germination, the seeds were treated using different concentrations of NAA (0%, 2%, 4%, 6%, 8%, 10%, 20%), GA3 (0%, 2%, 4%, 6%, 8%, 10%, 20%), and KNO3 (0%, 1%, 2%) solutions alone for six hours in beakers. Distilled water was used as a control for all treatments. Sixty seeds of *O. alpina* were used for each treatment with three replications, and the experiment was laid out on a completely randomized design (CRD). Seeds were sown uniformly on medium and kept moist. Continuous observation and recording of germination data were carried out at 7-day intervals up to 45 days after sowing. Also, the data on shoot number, shoot length (cm), root number, and root length (cm) were recorded after forty days of sowing.

For this experiment, the solutions of NAA, GA_3, and KNO_3 were prepared by weighing 100 mg and then dissolved in 100 ml of distilled water to have a 1:1 concentrated stock solution. During preparation, droplets of absolute alcohol were first added to dissolve the NAA solutes completely. Then, the required amounts of treatments were prepared for 100 ml of water by measuring the determined amount.

Germination Percentage, Mean Germination time, and Vigor Index

The Number of germinated seeds was recorded at a seven-day interval. Also abnormal, fungal-infected, and un-germinated seeds were considered non-viable. Accordingly, the germination percentage was calculated based on the counts of normal radicales in the seed embryo (FAO, 1985) using Eq. 1 below;

$$\text{Germination percentage (\%)} = \frac{\text{Total Germinated Seeds}}{\text{Total Sown Seed}} \text{ X } 100 \quad \text{Eq.1}$$

Similarly, assessments of mean germination time (MGT), germination speed or peak value (PV), and vigor index (VI) were also calculated by adopting Eq. 2 (Ellis and Roberts, 1981), Eq. 3 (Ayana *et al.*, 2012), and Eq. 4 (Ellis and Roberts, 1981, cited at Mavi *et al.,* 2010), respectively.

$$\text{Mean germination time (MGT)} = \frac{\Sigma Dn}{\Sigma n} \quad \text{Eq. 2}$$

Where n is the final germinated seeds on day D, and D is the total germination days.

$$\text{Germination speed/Peak value (PV)} = \frac{GP}{\Sigma Dn} \quad \text{Eq. 3}$$

Where PV = peak value, GP = germination percentage, Dn = total germination days.

$$\text{Vigor Index (VI)} = (\text{root length} + \text{seedling length}) \times GP \text{—Eq.4}$$

Data Analysis

The data were subjected to analysis of variance (ANOVA) using SAS 9.4 (SAS, 2008) as per standard procedures. In addition, REGWQ test at a 5% confidence interval was conducted for mean separation.

Results

Seed pretreatment effect on germination and shoot performance

The Effect of presowing treatments in Table 1 were highly significant ($p \leq 0.001$) in all studied parameters except NAA, which is highly significant ($p \leq 0.01$) for shoot number. In addition, KNO_3 is very highly significant for average shoot length, MGT, and VI parameters, and also highly significant at shoot number and average root length; however, it is non-significant for average root number, GP, and PV (Table 1).

Effect of NAA, GA_3, and KNO_3 Pretreatments on Seed Germination

Germination enhancement of *O. alpina* seeds treated with GA3, NAA, and KNO3 are shown in Table 2. GA_3 at 8% gave the highest (30%) germination, followed

Table 1: ANOVA summary on seed pretreatments for germination and shoot quality of *O. alpina*

Variation Source	DF	Shoot number	Av. shoot length	Av root number	Av root Length	GP	PV	MGT	VI
		MS	MS	MS	MS	MS	MS	MS	MS
NAA	6	1.19**	0.82***	0.17***	3.16***	124.32***	0.25***	1.65***	3990.38***
KNO_3	2	1.42**	1.16***	0.06ns	0.96**	8.47ns	0.04ns	1.91***	2507.78***
GA_3	6	4.98***	2.55***	0.68***	5.43***	184.42***	0.77***	19.51***	10775.25***
CV		23.18	16.32	19.04	16.77	13.69	16.04	10.76	15.60

Note: ns= Non-significant at P≤0.05, **= Significant at P≤0.01, and *** =Significant at P≤0.001

by 23% at 20%, and NAA at 6% and 8% produced the maximum germination percent, but not significantly different. Also, the lowest (5% and 10%) germination percentages were recorded when treated with 20% NAA and 10% GA_3. Moreover, the germination trends were continuously steady manner as the concentrations of GA_3 increased from 2% to 6%, increased to 8%, and then decreased discontinuously up to 20% concentration. On the other hand, with NAA, the germination percentage increased continuously until reaching a maximum of 6% and then decreased continuously up to 20% treatment.

Accordingly, KNO_3 gave the maximum germination percent (18.33%) at 2%, and the germination rate continued an increasing trend when KNO_3 increased up to 2% concentration (Table 2). In addition to germination percentage, MGT and PV parameters are also indicators of germination performance, and the vigor index (VI) indicates the germination potential leading to shoot emergence and growth (Table 2). The highest MGT and PV values were recorded in control (0%) and 8% GA_3, respectively. The presowing treatment improved the germination of *O. alpina* seeds compared with the control. The control scored the highest value for MGT but the lowest value for PV parameters (Table 2). The MGT trend showed a discontinuous decrease from 0% (T1) to all treatments but increased slightly when increasing the NAA concentration.

Effect of NAA, GA_3, and KNO_3 pretreatments on shoot performance

Results for the mean shoot and root length, number of roots, germinated seeds, and shoot emergence value are shown in Table 2. The highest shoot number, shoot length, and root length were recorded at 8% GA_3 which are not significantly different at 6% NAA and 20% GA_3 (Table 2). However, the highest root number was recorded at 20% GA_3 and 8% NAA, which are not significantly different with 4% and 10% GA_3, and 2-4% NAA concentration. Whereas, NAA produced well performed shoot number at 6% concentration.

KNO3 also produced better performance on all studied quality parameters of plantlets as compared with the control. Besides shoot number, the highest averages of shoot length, root number, and root length were recorded at 2% KNO_3 concentration. However, there was a slight radicle death rather than a complete change in shoot emergence (Table 2). In addition, the trends of phytohormones pretreatment showed a continuous increase in the average root length until it reached 8% concentrations of both GA_3 and NAA. But it was decreased continuously up to 20% concentration. Whereas the average shoot length showed a continuous increase until it reached 20% NAA concentration. However, the shoot number showed a discontinuous increasing trend until it reached to 8%, and then came back down up to 20% NAA.

Table 2: Mean Values for seed germination and shoot quality performance of *O. alpina*

Treat-ment	NAA (%)	KNO_3 (%)	GA_3 (%)	SN (mean±SD)	AvSL (mean±SD)	AvRn (mean±SD)	AvRL (mean±SD)	GP (mean±SD)	PV (mean±SD)	MGT (mean±SD)	VI (mean±SD)
T1	0	0	0	0.00±0.00^{e}	0.00±0.00^{h}	0.00±0.00^{d}	0.00±0.00^{h}	6.67±2.89^{f}	0.27±0.07^{f}	8.04±0.27^{a}	0.00±0.00^{i}
T2	0	0	2	2.67±0.58^{abc}	1.44±0.13^{def}	1.00±0.09^{c}	1.55±0.58^{def}	16.67±2.89^{cd}	0.92±0.14^{bcd}	1.40±0.09^{def}	48.95±3.39^{ef}
T3	0	0	4	2.33±0.58^{bcd}	2.43±0.11^{a}	1.25±0.05^{ab}	2.92±0.11^{ab}	16.67±2.89^{cd}	0.94±0.05^{bc}	1.41±0.23^{def}	88.98±14.71^{cd}
T4	0	0	6	1.67±0.58^{bcd}	2.09±0.64^{a-d}	1.11±0.19^{c}	2.61±0.40^{bc}	15.00±0.5^{ed}	0.77±0.20^{cde}	2.18±0.22^{c}	70.50±13.33^{de}
T5	0	0	8	4.00±0.08^{a}	2.63±0.33^{a}	1.17±0.14^{b}	3.43±0.54^{a}	30.00±0.5^{a}	1.88±0.10^{a}	0.71±0.26^{g}	181.65±10.67^{a}
T6	0	0	10	1.33±0.58^{cd}	0.88±0.13^{fg}	1.33±0.15^{ab}	0.33±0.06^{gh}	10.00±0.5^{ef}	0.72±0.24^{cde}	1.78±0.23^{cde}	12.08±0.81^{hi}
T7	0	0	20	3.00±1.00^{ab}	1.66±0.14^{b-e}	1.40±0.35^{a}	0.81±0.18^{fg}	23.33±2.89^{b}	1.29±0.28^{b}	1.01±0.09^{fg}	57.38±6.20^{ef}
T8	0	1	0	1.67±0.58^{bcd}	0.66±0.21^{g}	0.89±0.19^{c}	0.87±0.23^{fg}	15.00±0.05^{ed}	0.99±0.02^{bc}	1.94±0.18^{cd}	22.85±0.61^{ghi}
T9	0	2	0	3.00±0.08^{ab}	1.60±0.34^{b-e}	1.17±0.29^{b}	1.25±0.20^{ef}	18.33±2.89^{bcd}	1.13±0.09^{bc}	1.19±0.21^{efg}	51.49±3.56^{ef}
T10	2	0	0	1.67±0.58^{bcd}	1.06±0.29^{efg}	1.33±0.58^{ab}	1.28±0.11^{ef}	16.67±2.89^{cd}	0.73±0.17^{cde}	1.99±0.20^{cd}	39.72±13.71^{fg}
T11	4	0	0	2.00±0.08^{bcd}	1.68±0.11^{b-e}	1.28±0.07^{ab}	1.79±0.21^{de}	10.00±0.05^{ef}	0.51±0.02^{def}	1.12±0.11^{fg}	34.63±2.47^{fgh}
T12	6	0	0	3.00±0.09^{ab}	1.57±0.14^{c-f}	1.33±0.04^{ab}	2.56±0.40^{bc}	23.33±2.89^{b}	1.11±0.16^{bc}	1.47±0.15^{def}	96.05±11.80^{bc}
T13	8	0	0	2.33±0.58^{bcd}	2.03±0.41^{a-d}	1.50±0.10^{a}	3.35±0.14^{a}	21.67±2.89^{bc}	0.89±0.11^{bcd}	2.21±0.21^{c}	116.23±15.50^{b}
T14	10	0	0	2.33±0.58^{bcd}	2.34±0.14^{ab}	1.17±0.15^{b}	2.12±0.15^{cd}	16.67±2.89^{cd}	0.92±0.14^{bcd}	1.48±0.02^{def}	74.40±13.83^{cde}
T15	20	0	0	1.00±0.08^{de}	2.30±0.09^{abc}	1.00±0.10^{c}	0.20±0.08^{gh}	5.00±0.05^{f}	0.46±0.07^{ef}	3.31±0.50^{b}	12.50±0.80^{hi}
	CV			**23.18**	**16.32**	**19.04**	**16.77**	**13.69**	**16.04**	**10.76**	**15.60**

Note: Mean values in the same letter are not significantly different. NAA – Naphthalene acetic acid, KNO3 – potassium nitrate, GA3 – Gibberellic acid, SN – shoot number, AvSL – average shoot length, AvRn – average root number, AvRL – average root length, GP – germination percentage, PV – peak value, MGT – mean germination time, VI – vigorous index

Discussions

The effect of NAA, GA3, and KNO3 pretreatment on seed germination

Compared with the control, the germination percentage showed improvements in presowing treatments. GA_3, NAA, and KNO_3 enhanced the germination percent of the *O. alpina* seed when compared with the control. This germination improvement is not satisfactory for large seedling production of *O. alpina* seed. It might be due to the recalcitrant behavior of the seeds, which decline over time and are not viable after a month (Tesfaye *et al.,* 2022). This leads to explanation that the presowing treatments played a regulation role in the physiological activity of the embryo to improve seed germination. Igams and Diara (2017) reported that pretreatments improved germination speed, germination percentage, speed of seedling emergence, and reduced the number of abnormal seedlings by using GA_3 and KNO_3. GA_3 showed superior performance as compared to NAA and KNO_3, respectively. This evidence indicates that plant growth regulators have induced the intact genes responsible for germination to regulate the physiology of the embryo more than the nutritional value. Mensah *et al.,* (2020) reported the highest DNA concentration on flax seed radicals generated from GA_3 treatment than that of KNO_3 and the control treatment, which provided an equally low DNA concentration. This is concrete evidence that plant growth regulators can induce the genes to replicate that are responsible for cell division and development.

Although the exogenous plant growth regulator treatments promote the germination percentage of *O. alpina* seeds, the results are still very low and unsatisfactory in both GA_3 and NAA pretreatments. This might be due to the cases related to the recalcitrant behaviors of *O. alpina* seeds, which become nonviable after long storage. On the other hand, there might be a limited availability of endosperms, which have been used as an embryo food source until they can develop their own shoot with leaves to produce their food through photosynthesis. Moreover, it might also be due to the concentration rate, soaking duration differences and the type of exogenous hormone as compared with the previous research (Gashi *et al.,* 2012; Karim and Variyani, 2016; Emem *et al.,* 2017; Rout *et al.,* 2017; Singh, 2020). These authors employed a high dose of GA_3 (100 to 1000 ppm) and NAA (50 to 200 ppm) with a soaking duration from 6 to 72 hours at different species of seed and obtained satisfactory germination percentages. However, there has not been any available information on the species used in this study. Shuai *et al.,* (2017) also reported that abscisic and gibberellic acids were the key regulators of seed germination, and their ratios determined the success of germination. In addition, the trends of germination capacity showed a continuously steady manner but increased when approached to 8%, then showed a discontinuous decreased manner up to 20% concentration. In this unstable manner, the fungal contamination of the seed might have contributed because the fungal-infected seeds were avoided immediately and not counted if they did not have a radicle before being infected. Whereas, NAA shows a continuously increased trend until it reaches its peak and then continuously decreases.

KNO_3 also promoted germination percent; however, it scored the least as compared to the results recorded by GA_3 and NAA. This evidence suggests that plant growth regulators play a significant role in regulating the embryo's metabolic pathways. In addition, the germination rate remained on an increasing trend, which indicates that there needs to be a further increase in KNO_3 rates to obtain the optimum ratio that produces the maximum germination percentage. However, there is not much more dosage of KNO_3 reported than that of the ratios used in this research. This indicates that it might be needed in a low dosage amount other than the high dosage that leads to abnormal germination and shoot emergence, but it needs to be proven.

In addition to germination percentage, MGT and PV parameters are also indicators of germination performance. Also, the vigorous index (VI) indicates the germination potential that can change to shoot emergence and growth (Table 2). The presowing treatments improved the germination capacity of *O. alpina* seed as compared with the control, which scored the highest value in MGT and the lowest value in PV (Table 2). The trends of MGT showed a discontinuous decrease but slightly increased when the NAA concentration increased. This indicates that the plant growth regulators and nutrients adversely affected the seed germination capacity, but the high dose of NAA influenced the germination percentage by regulating the metabolism of the seed embryo. Among the treatments, GA3 showed higher seed germination

improvement than others by scoring a high value on germination percentage, peak value, and vigorous index but the lowest MGT because it is reciprocally related to germination percentage and peak value.

Generally, the germination percentage is still unsatisfactory. However, the obtained information indicates that the use of exogenous phytohormones regulated and enhanced the germination of *O. alpina* seeds that have low viability and germination during storage. Hence, soaking *O. alpina* seeds using GA_3 and NAA separately for 6 hours produced a good germination percentage. In addition, it is also advisable to use KNO_3 in combination with either GA_3 or NAA to boost the germination potential rather than using KNO_3 alone.

Effect of NAA, GA_3, and KNO_3 pretreatments on plantlets performance

The shoot emergence and its parameters showed a significant effect as affected by the use of GA_3, NAA, and KNO_3 to improve the plantlet's quality as compared with the control, which had no successful shoot emergence. The presence of these presowing treatments also improved shoot emergence from the germinated seed by reducing death. However, there was still no complete tackling of radicle death. Utami and Hariyanto (2016) also obtained the highest seed germination percentage and the formation of shoots using MS media for in vitro germination of *Dendrobium antennatum.*

The shoot number, shoot length, and root length are high in 8% GA_3. However, they responded differently to NAA, which highly promoted root numbers. This evidence indicates that GA_3 has not been induced as an intact gene responsible for adventitious root induction, but NAA has a role in stimulating cell division and development. That is why NAA induces root initiation and development. Because GA_3, as a growth hormone, has a role in enhancing seedlings' development and growth, besides other functions such as stimulating cell development and elongation. According to this physiological regulation difference of phytohormones, GA_3 showed better performance on shoot number, shoot length, and root length than NAA, which could potentially perform on only root number. Plant growth regulators such as GA_3 and NAA play a pivotal role in promoting seedling performance on different tree species, as reported in (Dev *et al.,* 2020; Maku *et al.,* 2014; Afzal *et al.,* 2005). Hence, it is potentially recommended to use GA_3 to enhance seed germination, shoot emergence, and plantlet quality rather than auxins and KNO_3, unless the interaction between GA_3 and KNO_3, which is highly recommended.

Furthermore, KNO_3 also performed well in all studied shoot quality parameters as compared with the control, but there was a slight radicle death rather than a change to complete shoot emergence (Table 2). In addition, it recorded the lowest value as compared with the phytohormones employed as presowing treatment. This indicates that KNO_3 has not had a key role in the regulation of embryogenic and plant physiology like that of phytohormones, but it has a nutritional value that somehow stimulates metabolic activity.

Furthermore, the trends of phytohormones pretreatment showed that the average root length continuously increased until it reached pick and then continuously decreased at both GA_3 and NAA. Whereas the average shoot length continued a continuously increasing trend in NAA, and the shoot number showed a discontinuous increased trend until it reached up to a slight pick point at 6% NAA and 8% GA3 (Table 2).

Conclusion and Recommendations

Oldeania alpina (K. Schum) Stapleton seed has not produced satisfactory germination and shoot emergence performance without any treatment. To tackle this challenge, different seed pre-treatments were evaluated to induce germination and shoot vigor. Presowing treatments GA3, NAA, and KNO3 for 6 hours, improved the germination and plantlet performance. Among various treatments, GA_3 significantly improved all studied parameters of germination and plantlet performance, followed by NAA and KNO_3, respectively. The highest germination percentage (30%, 23%, and 18.33%) was recorded for 8% GA_3, 6% NAA, and 2% KNO_3, respectively. Furthermore, GA_3 also showed higher performance on shoot number and length, besides promoting germination performance than others. Hence, 8% GA_3 is recommended as a presowing treatment to enhance germination and plantlet performance, followed by 6% NAA and 2% KNO_3, respectively.

Based on this study, the following future lines are given:

- In this study, fungal contamination that leads

to low germination capacity was a major challenge; hence, this study may be repeated using fungal disinfection.

- Also, this study used 3-months-old seeds stored at 5°C; however, further research should be conducted on seeds stored at different storage temperatures and periods.
- Additionally, this study did not include other cytokinins hormones, so it would be worth trying BAP, KIN, and others.

Acknowledgements

The authors would like to thank Ethiopian Forestry Development for their financial funds and other supports. Also, the authors give heartfelt gratitude to Central Ethiopia Forestry Development Center for its organized Tree Seed Quality Control Laboratory and other support. Finally, the authors appreciate all the Seed Research Project team, and laboratory technicians, for your unreserved help in all activities and data collection.

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