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The effect of GA3, NAA, and KNO_3 on *in-vitro* seed germination and plantlets performance of Highland Bamboo (*Arundinaria alpina)*, South-West Ethiopia

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Abstract

Propagation through seed is commonly used and cost effective to propagate tree plants if it is available and does not has different germination and shoot emergence difficulties. However, *Arundinaria alpina* seed has low germination performance and short storage period due to its mid recalcitrant behavior. Therefore, this research was initiated to evaluate the effects of pre-treatments on three months stored seed to improve germination and growth of shoots. The collected seeds were processed and brought to the laboratory. The analysis of variance revealed that the pretreatment factors NAA, GA_3, and KNO_3 were very highly significant ($P \leq 0.001$) for all studied parameters except NAA was highly significant($P \leq 0.01$) for shoot number, and also KNO3 at shoot number and root length. However, KNO3 was non-significant at root number, GP, and PV. In this result, GA_3 gave the highest (30%) germination percentage and shoot number at 8% and then followed by 23% at 20%, whereas NAA gave the highest (23%) at 6%, followed by 22% at 8%, but there was no somehow effective on KNO_3 treatment. the lowest (10% & 5%) germination percentage was recorded at 10% GA_3 and 20% NAA, respectively. In GA_3, the highest shoot number, average of shoot length and root length were recorded at 8% whereas NAA produced the highest average root number and length at 8%, and average shoot length at 10% and 20%, but at 6% for shoot number. This concluded that the use of GA_3 or NAA as a pre-treatment to enhance the germination and shoot quality of *A. alpina* seed is recommended. Also, surface disinfection should be practiced to avoid infections.

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Introduction

In some tree species, however, there is a challenge to use seed as a valuable source for propagation due to dormancy, hard seed coat, low longevity, and others. Among those, *Arundinaria alpina* (K. Schum) is one that has a challenge to use its seed as a valuable source for enough seedlings production because of long flowering cycles, poor storage and short viability of seeds due to its recalcitrant behavior, and the presence of disease and pests, etc. (Bahru *et al.,* 2015; Ayana *et al.,* 2014 & 2012). Seed germination has also been varied between species and seed lots, and even within seed lots because seeds are a living biological end product and their behavior can be determined by the interaction of genetic and environmental effects (Bahru *et al.,* 2015; Fredrick *et al.,* 2015; Derero *et al.,* 2012; Loha *et al.,* 2006; Mamo *et al.,* 2008 &2006). In addition to the average germination capacity and period, the weight and sizes of the seed which can be correlated positively with seed mass also strongly influenced by the genotype and agro-ecology of the mother tree (Carles *et al.,* 2009; Norden *et al.,* 2008).

Moreover, seed longevity is a crucial behavior to low-cost production besides maintaining the quality of seedlings. However, the *A. alpina* seed longevity is seriously very short and shows low germination performance within a month. Hence, different pretreatment of seed are needed and recommended to improve this low germination and shoot quality performance. Among the seed pretreatments, plant growth hormone and nutrient have been commonly used for different plant species, especially for seeds that become physiologically dormant and low viability within short period to enhance germination capacity and shoot performance (Dev *et al.,* 2020; Khodadadi, *et al.,* 2018; Jyoti *et al.,* 2016; Maku *et al.,* 2014; Cárdenas *et al.,* 2013; Gashi *et al.,* 2012; Afzal *et al.,* 2005). Studies of genetics and physiology have shown vital roles of the plant hormones, Abscisic, and Gibberellic acid, in the regulation of dormancy and germination (Kornneef *et al.,* 2002). Among the plant growth regulators, gibberellin is one of the major endogenous factors that enhance germination (Tuan *et al.,* 2018).

Even if using pretreatments to enhance germination and shoot quality of *A. alpina* seed is remarkably important to speed up bamboo forest development endeavors, there is no studied evidence on the priming of seeds using plant growth regulators and nutrients. Therefore, this study was initiated to evaluate the effect of plant growth regulators and nutrients on germination enhancement and shoot development performance of *A. alpina* seed to pace nursery seedlings production.

Material and methods

Study Area Description

The source seed was found from the storage in the seed quality laboratory of Central Ethiopia Environment and Forest Research Center (CE-EFRC). It was collected from the Desta site of Adeyo district in Kefa Zone, South West Ethiopia. It is far about 530km distance from Addis Ababa and lies between 7o 8' to 7o 26'N latitude and 36o 15' to 36o 50'E longitude. The elevation ranges from 500 to 3000m above sea level.

This study was conducted in the Tree Seed Quality Control laboratory of CE-EEFRC, Gurd-sholla, Addis Ababa; it lies between 9◦ 01′ 04′′ latitude and 38◦ 49′ 06′′ longitude, and also the elevation is 2377mas. This laboratory was established in 1975 in the forest sector, which is now well designed and organized in all aspects for tree seed quality tests, and it is the only representative of tree seed quality tests in the country.

Determination of Seed Moisture Content

After three months of storage at room temperature, pure seed samples were taken from stored seeds, and then the moisture content of *A. alpina* seeds was determined following the methods used by FAO (FAO, 1985). This seed has 88.4% purity with the size of 0.47cm and 0.25cm for seed length and width, respectively (Getnet *et al.,* 2021).

For moisture content analysis, two samples of 2mg each were taken from the total seed lot of A. alpina, and then the weighed samples were subjected to automatic moisture content determination machine.

This advanced moisture analyzer machine was worked both the seed weighing and drying process, finally it was given the moisture result automatically within 15 to 30 minutes.

Effect of Pretreatments on Germination Capacity and Plantlets Quality of A. alpina Seed

The enhancement of germination capacity of *A. alpina* seed which reduced within a short period was conducted by using metabolic activator treatment. The effect of presowing treatments on the germination of *A. alpina* seeds was evaluated by conducting different concentrations of plant growth regulators and inorganic nutrients. For the determination of pretreatment effect on seed germination, *A. alpina* seeds were treated by the different concentration of NAA (2%, 4%, 6%, 8%, 10%, 20%), GA3 (2%, 4%, 6%, 8%, 10%, 20%), and KNO3 (1%, 2%) solutions alone for six hours in the beaker by using only water solution (0%) as a control all. Sixty seeds of *A. alpina* were used for each treatment with three replications, and then the experiment was laid out using a completely randomized design (CRD). The optimum moisture was maintained by spraying equally throughout treatments using distilled water slightly to avoid maximum wet as this would have a negative effect on seed germination (Embaye, 2003). Finally, the germinated seed data was recorded in five-day intervals up to the closing date, and also the data of shoot number, shoot length (cm), root number, root length (cm) were recorded after forty days of sowing.

For this experiment, the solutions of NAA, GA3, and KNO3 were prepared by weighing 100mg and then dissolved in 100ml distilled water to have a 1:1 concentrated stock solution. During preparation, droplets of absolute alcohol were added to dissolve the NAA solutes completely. Then, the required amount of treatments was prepared for 100ml water by measuring the determined amount using an appropriate measuring cylinder.

Germination Percentage, Mean Germination time, and Vigor index

Seeds were sown uniformly and watered as needed to keep moist but not wet. Then, continuous attendant and germination data records were taken place. The number of germinated seeds was recorded through seven days intervals, and also abnormal seeds, seeds infected with fungus, and not germinated seeds were considered as non-viable. Accordingly, germination percentage was calculated based on the counts of normal radicals from seed embryo (FAO, 1985) using eq. 1 below:

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

Similarly, Assessment of mean germination time (MGT) was also made by adopting eq. 2 (Ellis and Roberts, 1981)

$$MGT = \frac{\sum Dn}{\sum n} \; eq\ (2)$$

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

Germination speed/Peak Value

It is calculated by adopting eq.3 from Ayana *et al.* (2012) as follows below

$$PV = \frac{GP}{\Sigma \text{Dn}} \; eq\ (3)$$

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

Vigor index (VI)

VI was calculated by eq.4 adopted from the Ellis and Roberts (1981, cited at Mavi *et al.,* 2010). VI = (root length + seedling length) × germination percentage (4)

Data Analysis

The collected data were subjected for analysis of variance (ANOVA) using SAS 9.4 (SAS, 2008) as per standard procedures. In addition, multiple comparison of REGWQ multiple range test at 5% confidence interval was used for mean separation. The data was also evaluated by using tables and graphs.

Results and discussion

Analysis of Seed Pretreatment Effect on Germination and Plantlets Performance

According to the analysis of variance result (Table 1), the effect of GA3 and NAA were very highly significant ($p \leq 0.001$) in all studied parameters except NAA that was highly significant ($p \leq 0.01$) at shoot number. In addition, KNO3 was also shown very highly significant at average shoot length, MGT, and VI parameters, and also highly significant at shoot number and average root length, however, it was non-significant at average root number, GP, and PV (Table 1).

Table 1. ANOVA summary on presowing treatment for seed Germination and Shoot Quality of *A. 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***
KNO3	2	1.42**	1.16***	0.06ns	0.96**	8.47ns	0.04ns	1.91***	2507.78***
GA3	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

The Effect of NAA, GA_3, and KNO_3 Pretreatment on Seed Germination Percentage

The germination percentages of *Arundinaria alpina* seed, which was stored at ambient temperature for 3 months, treated with GA3, NAA, and KNO3 are presented below (Table 2; Fig 1 & 2). Compared with the control, the presowing treatments showed significant promoting effects on germination percentage. The concentrations of GA3, NAA, and KNO3 enhanced the germination capacity of *Arundinaria alpina* seed when compared with the control treatment which had 6.67%. This leads to explain that the presowing treatments had important regulation on the physiological activity of the embryo to improve seed germination. IGAMS and Diara (2017) reported that pretreatments were improved germination speed, germination percentage, speed of seedlings emergence, and reduced the number of abnormal seedlings by using GA3 and KNO3. Across the treatments, GA3 showed superior performance followed by NAA and KNO3, respectively. This evidence indicated that plant growth regulators have induced the intact genes responsible for germination to regulate the physiology of the embryo than that of the nutritional value. Mensah *et al.* (2020) reported the highest DNA concentration on flax seed radicals generated from GA3 treatment than that of KNO3 and control treatment which provided equally very low DNA concentration.

GA3 at 8% gave the highest (30%) germination capacity and followed by 23% at 20% whereas NAA produced the maximum germination capacity at 6% and 8% which were not significantly different. However, still, the germination percentage of *A. alpina* was very low and unsatisfactory; this might be due to the case related to recalcitrant seeds of *A. alpina* which could become nonviable for long storage or the limited availability of endosperms which have been used as an embryo food source until it could emerge shoot with leaves, and then produced their food through photosynthesis. Moreover, it might also be due to the concentration rate and soaking duration difference, 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). The authors have employed a high dose of GA3 (100 to 1000 ppm) and NAA (50 to 200 ppm) with a soaking duration from 6h to 72hrs at different species seed, and obtained satisfactory germination percentage but not any available information released on the species used in this study. Shuai *et al.* (2017) also reported abscisic acid and Gibberellic acid were the key regulators of seed germination, and their ratios determined the successfulness of germination.

Also, the lowest (5% & 10%) germination capacity was recorded at 20% NAA and 10% GA3 treatment. In addition, the trends of germination capacity showed continuously steady when the concentrations of GA3 increases from 2% to 6%, however, it was increased at 8%, and then discontinuously decreased to 20% concentration treatment. This disturbing result might

be contributed by the fungal contamination of the seed because the fungal infected seeds were avoided immediately, and not counted if they could not have radicle before being infected. Whereas in NAA, the germination capacity was continuously increased till reached to peak at 6%, and then continuously decreased to 20% treatment.

Accordingly, KNO3 provided the maximum (18.33%) germination capacity at 2%, however, it was scored the least as compared to the results recorded by GA3 and NAA. This evidenced that plant growth regulators had a significant role to determine the metabolic pathways of the embryo. In addition, the germination rate remained in an increasing trend which indicated that it needs a further increase of KNO3 rates to obtain the optimum ratio that produces maximum germination percentage. However, there is not much more dosage of KNO3 report than that of the ratios used in this research. This indicates that it may need in low dosage amount other than the high dosage that leads to abnormal germination and shoot emergence, but it needs to be proved.

In addition to germination percentage, mean germination time (MGT) and peak value (PV) parameters are also the important indicators of germination performance. The highest MGT and PV were recorded on the control (0%) and 8% GA3, respectively (Table 2). Presowing treatments were improved the germination capacity of A alpina seed as compared with the control which scored the highest value in mean germination time but lowest in peak value parameters (Fig 2; table 2). The trends of MGT were shown discontinuously decrease from 0% (T1) to any treatments but slightly increased when the NAA concentration increased. This indicates that the plant growth regulators and nutrient were adversely affected the seed germination capacity, but the high dose of NAA influenced the germination percentage by regulating the metabolism of seed embryo. Among the treatments, GA3 showed higher seed germination improvement than others by scoring high value on germination percentage, peak value, and vigorous index but the lowest MGT because it has reciprocally related with germination percentage and peak value.

Generally, although the germination percentage is still unsatisfactory, the obtained information indicated that the use of exogenous phytohormones regulated and enhanced germination of *A. alpina* seeds that have a weak performance during storage. Hence, soaking of *A. alpina* seeds using GA3 at 8% and NAA at 6%, separately for 6hrs were recommended, and helped to enhance germination capacity. In addition, it is also advisable to use 2% KNO3 in combination with either GA3 or NAA to boost the germination potential rather than using KNO3 alone.

Table 2. Mean Values for seed germination and shoot quality of *Arundinaria alpina* Species.

NAA (%)	KNO3 (%)	GA3 (%)	SN (mean±SD)	AvSL (mean±SD)	AvRn (mean±SD)	AvRL (mean±SD)	GP (mean±SD)	PV (mean±SD)	MGT (mean±SD)	VI (mean±SD)
0	0	0	0.00±0.00e	0.00±0.00h	0.00±0.00d	0.00±0.00h	6.67±2.89f	0.27±0.07f	8.04±0.27a	0.00±0.00i
0	0	2	2.67±0.58abc	1.44±0.13def	1.00±0.00c	1.55±0.58def	16.67±2.89cd	0.92±0.14bcd	1.40±0.09def	48.95±3.39ef
0	0	4	2.33±0.58bcd	2.43±0.11a	1.25±0.05ab	2.92±0.11ab	16.67±2.89cd	0.94±0.05bc	1.41±0.23def	88.98±14.71cd
0	0	6	1.67±0.58bcd	2.09±0.64a-d	1.11±0.19c	2.61±0.40bc	15.00±0.00ed	0.77±0.20cde	2.18±0.22c	70.50±13.33de
0	0	8	4.00±0.00a	2.63±0.33a	1.17±0.14b	3.43±0.54a	30.00±0.00a	1.88±0.10a	0.71±0.26g	181.65±10.67a
0	0	10	1.33±0.58cd	0.88±0.13fg	1.33±0.15ab	0.33±0.06gh	10.00±0.00ef	0.72±0.24cde	1.78±0.23cde	12.08±0.81hi
0	0	20	3.00±1.00ab	1.66±0.14b-e	1.40±0.35a	0.81±0.18fg	23.33±2.89b	1.29±0.28b	1.01±0.09fg	57.38±6.20ef
0	1	0	1.67±0.58bcd	0.66±0.21g	0.89±0.19c	0.87±0.23fg	15.00±0.00ed	0.99±0.02bc	1.94±0.18cd	22.85±0.61ghi
0	2	0	3.00±0.00ab	1.60±0.34b-e	1.17±0.29b	1.25±0.20ef	18.33±2.89bcd	1.13±0.09bc	1.19±0.21efg	51.49±3.56ef
2	0	0	1.67±0.58bcd	1.06±0.29efg	1.33±0.58ab	1.28±0.11ef	16.67±2.89cd	0.73±0.17cde	1.99±0.20cd	39.72±13.71fg
4	0	0	2.00±0.00bcd	1.68±0.11b-e	1.28±0.07ab	1.79±0.21de	10.00±0.00ef	0.51±0.02def	1.12±0.11fg	34.63±2.47fgh
6	0	0	3.00±0.00ab	1.57±0.14c-f	1.33±0.04ab	2.56±0.40bc	23.33±2.89b	1.11±0.16bc	1.47±0.15def	96.05±11.80bc
8	0	0	2.33±0.58bcd	2.03±0.41a-d	1.50±0.10a	3.35±0.14a	21.67±2.89bc	0.89±0.11bcd	2.21±0.21c	116.23±15.50b
10	0	0	2.33±0.58bcd	2.34±0.14ab	1.17±0.15b	2.12±0.15cd	16.67±2.89cd	0.92±0.14bcd	1.48±0.02def	74.40±13.83cde
20	0	0	1.00±0.00de	2.30±0.00abc	1.00±0.00c	0.20±0.00gh	5.00±0.00f	0.46±0.07ef	3.31±0.50b	12.50±0.00hi
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.

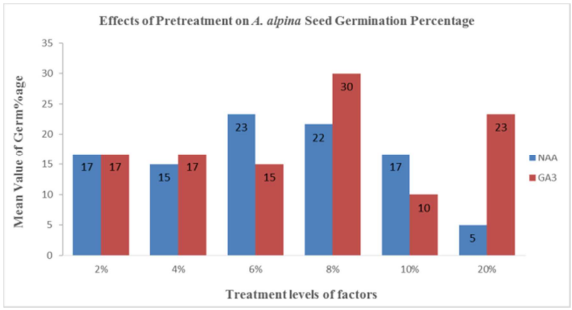

Fig. 1. Effect of NAA and GA_3 pretreatment on in vitro *A. alpina* seed germination capacity.

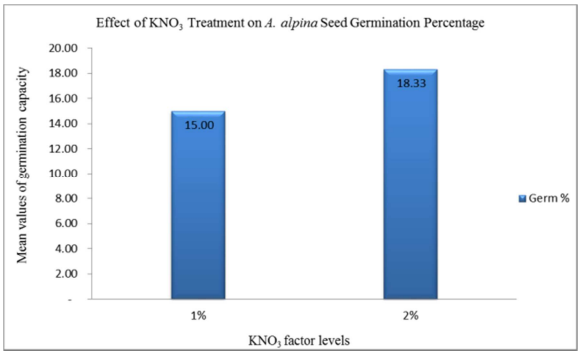

Fig. 2. Effects of KNO_3 pretreatment on in vitro *A. alpina* seed germination capacity.

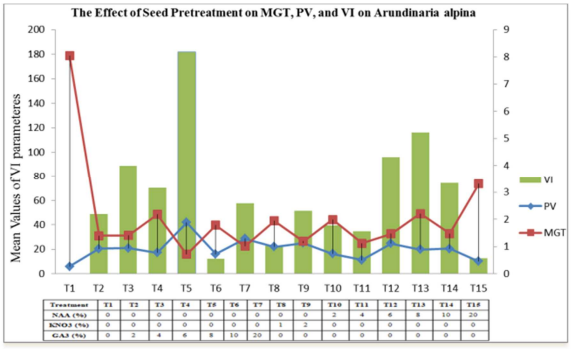

Fig. 3. The effect of NAA, KNO3, and GA3 on MGT, PV, and VI performance.

The Effect of NAA, GA_3, and KNO_3 Pretreatment on Plantlets Performance

The results regarding the average of shoot length, root length, root number, and germinated seed and its shoot emergence value are presented in (Table 2, and Fig 4, 5, & 6). This result revealed that there was a significant effect as affected by the use of GA3, NAA, and KNO3 to improve plantlets' quality as compared with the control that had no successful shoot emergence. The presence of these presowing treatments also improved the shoot emergence from the germinated seed by reducing death, however, still, there was no tackled the radicles death completely. Utami and Hariyanto (2016) also obtained the highest seed germination percentage (92.2) and the formation of shoots (51.4%) using MS media in vitro germination of Dendrobium antennatum.

In GA3, the highest shoot number, average of shoot, and root length were recorded at 8% concentration and followed by 20% GA3, 2% KNO3, and 6% NAA on shoot number but 10% and 8% NAA for average of shoot and root length, respectively (Table 2). Also, 4% of GA3 and 8% of NAA were produced average shoot and root length, respectively, that are not significantly different from 8% GA3. Similarly, NAA also gave the highest (3.36cm, 1.5) average of root length and root number at 8% concentration and followed by 2.56cm and 1.33cm at 6% concentration, respectively. However, the highest average shoot length was recorded differently at 10% and 20% NAA concentration (Table 2). This evidence indicated that the GA3 has not been induced intact gene responsible for adventitious root induction, but NAA has a role to stimulate cell division and development that is why it induces root initiation and development. Because GA3, as a growth hormone, has a role to enhance seedlings growth besides other functions such as stimulate cell development and elongation. According to this physiological regulation difference of phytohormones, GA3 showed better performance on shoot number, shoot length, and root length than NAA which was potentially performed on only root number. Plant growth regulators such as GA_3 and NAA play a pivotal role to promote seedlings performance on different tree species as reported in (Dev *et al.,* 2020; Maku *et al.,* 2014; Afzal *et al.,* 2005). Hence, it is potentially advisable to use GA3 to enhance seed germination, shoot emergence, and plantlets quality rather than auxins and KNO3, unless the interaction use of GA3 and KNO3, which is highly recommended.

Furthermore, KNO3 was also performed in all studied plantlets quality parameters in addition to the number of germinated seed and emerged shoot as compared with the control; however, it was revealed

the least seed germination and shoot quality parameters improvement as compared with the phytohormones employed as presowing treatment. This indicated that the KNO3 has no a key role in the regulation of embryo and plant physiology like that of phytohormones but it has a nutritional value that stimulates somehow metabolic activity. The highest average of shoot length, root number, root length, and also shoot number was recorded at 2% KNO3 concentration, however, there was slightly radicle death rather than changed to shoot emergence completely (Fig. 6).

In addition, the trends of phytohormones pretreatment showed that the average root length was increased continuously until it reached the turning point at 8% concentration of both GA3 and NAA, and continuously decreased down to 20% concentration. Whereas the shoot number showed continuously increased till it reached to 20% concentration in NAA, but the average root number was slightly showed a turning point at 8% concentration in continuous increased and decreased manner.

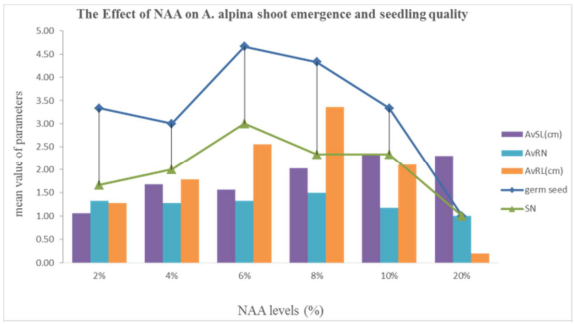

Fig. 4. The effect of NAA on in vitro germinated *A. alpina* plantlets performance.

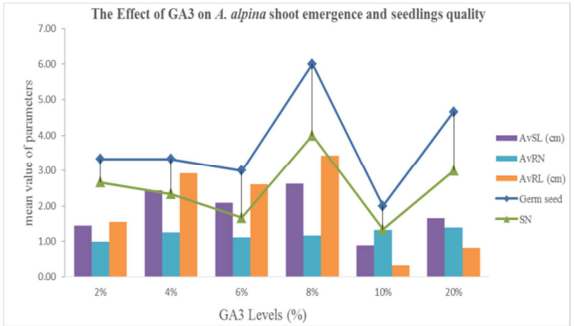

Fig. 5. The effect of GA_3 on in vitro germinated *A. alpina* plantlets performance.

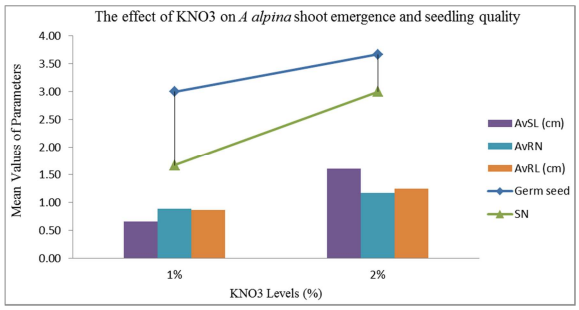

Fig. 6. The effect of KNO_3 on in vitro germinated *A. alpina* plantlets performance.

Conclusion and recommendations

Highland bamboo species (*Arundinaria alpina* L) seed has not been produced satisfactory germination and shoot emergence performance due to its immediate recalcitrant behavior. To confront this challenge, different seed pretreatments were evaluated to enhance the germination capacity and shoot emergence to reduce the remarkable germination declining. Hence, the PGRs and nutrient treatment for 6hrs affected the germination and plantlets performance. Among those, GA3 significantly improved all studied parameters of germination and plantlets performance followed by NAA and KNO3, respectively. The highest germination percentage (30, 23, and 18.33%) was recorded at 8% GA3, 6% NAA, and 2% KNO3, respectively. Furthermore, GA3 also showed higher performance on shoot number, and shoot length besides to promote germination than others. Hence, GA3 is strongly advised to enhance germination capacity and shoot emergence performance as seed pretreatments, followed by NAA and KNO3, respectively.

Based on this study result the following recommendations are given

- In this study, fungal contamination was a big challenge which might also be led low germination capacity, so that this study should be repeated using fungal disinfection.
- Also, this study was used 3 months stored seed at ambient temperature, but research should be conducted on different storage temperature and period.
- In addition, this study did not include other cytokinins hormones so that it should be observed using BAP, KIN, and others.

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