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Effect of Gibberellic Acid and Germination Medium on Kiwifruit Seed Germination

Tshering Yangchen[f], Tshetrim[g], Lungki[f], Loday Phuntsho[f]

ABSTRACT

In Bhutan, cultivation of exotic commercial Kiwifruit fruit is new though wild kiwifruit is found aplenty. There is a high scope for kiwifruit cultivation as it is suitable in a wide range of areas in the country. It can also be grown organically due to the lack of any known serious pest or disease. Fittingly, kiwifruit is counted among the most potential high-value fruit crops in the country. Its cultivation is gaining popularity in the current times and it would consequently increase the demand for kiwifruit seedlings. Kiwifruit seeds exhibit physiological seed dormancy which hampers kiwifruit seedling production. Hence, a study was conducted in 2019 and 2020 at ARDC, Wengkhar. In the study, the combined effect of GA3 and growth medium on kiwifruit seed germination was studied using eight treatments with three replications laid out in a Completely Randomized Design. The study aimed to find out the germination parameters like germination percentage, relativized percentage, mean germination time, time taken for 10% germination, and time taken for 25% germination. According to the study, Cocopeat + 1500 ppm GA3 gave the best germination percentage at 51% followed by Biochar + 1500 PPM at 39% while rice bran and compost exhibited a low germination percentage. Overall, the mean germination time was found to be 50 days. The highest relativized percentage of 63 % was observed in Cocopeat + 1500 ppm GA3. As for the time taken to achieve 10% and 20% germination, those treatments with 1500 GA3 application had an advantage of approximately eight days over the others. Overall, considering the average weighted effect of germination percentage and time, Cocopeat + GA3 1500 PPM gave the best result followed by Biochar + GA3 1500 PPM and Cocopeat + GA3 0 PPM. Hence, the study indicates that GA3 treatment in combination with an appropriate germination medium can significantly enhance seed germination in kiwifruit.

Keywords: *Kiwifruit germination; GA3; Growing medium; Germination percentage; Mean germination time*

1. Introduction

Kiwifruit (*Actinidia deliciosa*) is an edible berry of a woody vine. It is native to southwestern China. China is the global leader in kiwifruit production accounting for half of the total

Corresponding author: tsheringy@moaf.gov.bt
[f] Agriculture Research and Development Centre- Wengkhar, Department of Agriculture, Ministry of Agriculture and Forests
[g] Agriculture Production Division, Department of Agriculture, Ministry of Agriculture and Forests

worldwide production (Ferguson, 2015). In Bhutan, cultivation of exotic kiwifruit is comparatively recent (Gyeltshen, 2011) even though wild kiwifruit is found aplenty. Kiwifruit can be grown at an altitude ranging from 1400 to 2500 m above sea level. Its cultivation is suitable for a vast range of areas in the country and it can be grown under organic management (Phuntsho, 2010) as incidences of major pests and diseases are yet to be observed. In recent times, kiwifruit cultivation is gaining attraction in the country due to its high value in the market. It can cost up to Nu.250 (3.38 USD) for six medium-sized kiwifruits. Consequently, the demand for kiwifruit seedlings is increasing. Seedling production through grafting and cuttings are two modes of propagation for kiwifruit (Luh & Wang, 1984). Grafting on seedling rootstocks is preferred over propagation through cuttings for its better root system (Celik, Zenginbal, & Özcan, 2006). However, seed dormancy is prevalent in kiwifruit seed germination. Seed dormancy can be defined as the inability of a viable seed to germinate in the given environment (Bewley, 1997). Physiological germination is common in seeds of angiosperms and it is the predominant form of dormancy in seed banks of temperate fruits (Finch-Savage & Leubner-Metzger, 2006). For that reason, the germination rate of kiwifruit seeds is low (Maghdouri et al., 2021). These contribute to the difficulty in kiwifruit seedling production, and thus the issue of higher prices.

Various approaches such as GA3 application, stratification, and scarification can help in obtaining greater seed germination (Sekhukhune, Nikolova, & Maila, 2016). Likewise, other growth hormones play an important role in seed germination. Dormant seeds can germinate under the influence of ethylene by impeding the adverse effect of ABA on seed germination; radical growth in seeds is affected by ethylene and gibberellins where gibberellins are considered to have the most significant influence (Miransari & Smith, 2014). IAA priming was also found to stimulate seed germination by aiding in the production of useful endogenous phytohormones and hindering the production of inhibitory phytohormones (Zhao et al., 2020).

Numerous other studies showed positive influence of GA3 on seed germination. GA hormone works to trigger the transition from embryo to seedling; GA signaling aids in auxin-induced root growth and development of seeds (Hauvermale & Steber, 2020). GA interacts with ABA and environmental conditions to break dormancy (Kucera, Cohn, & Leubner-Metzger, 2005). Hence GA3 treatment alleviates seed germination and shortens the mean germination time (Bishwas et al.).

Similarly, growing media plays an important role in germination through effective root establishment. Growing media provides nutrients and influences the quality of roots produced (Shanker, Misra, Topwal, & Singh, 2019). The use of appropriate rooting medium facilitates proper aeration and drainage while the use of inappropriate substrate hinders the efficacy of water absorption (Miri-Nargesi, Sedaghathoor, & Environment, 2015). Currently, the use of growing media like biochar is picking up speed in Bhutan (Tamang, Dorji, & Dorji, 2020) and it has been released as a technology by the Technology Release Committee (TRC) of the Department of Agriculture, Ministry of Agriculture and Forests on 29 May 2020.

Therefore, this study assessed the effect of different growing media and gibberellic acid application in enhancing the germination percentage of kiwifruit seeds.

The experiment was carried out under greenhouse condition at ARDC Wengkhar which is located at 1708 m above sea level (27°16'14'' N; 091°16'19''E), Mongar, Bhutan. Seeds were manually extracted from well-ripened fruits of Wengkhar green cultivar harvested from the research block of ARDC Wengkhar. Extracted seeds were washed and air-dried.

2. Materials and Method

2.1 GA3 treatment

Seeds were soaked in 1500 ppm GA3 solution (7.3 pH) for 24 hours before the sowing time. As for the control treatments, seeds were soaked in distilled water (7.3 pH) for the same duration and kept in the laboratory at room temperature.

2.2 Experimental design

A two-factor factorial Completely Randomized Design with eight treatments and three replications was used. The factors comprised four growth mediums (Coco peat, Biochar, Rice bran, and Compost) and Gibberellic acid applications (0 ppm and 1500 ppm). In each treatment, 100 kiwifruit seeds were used as a unit of study. The treatments used were as follows:

1. T_1- Biochar + GA3 0 ppm
2. T_2- Biochar + GA3 1500 ppm
3. T_3- Coco peat + GA3 0 ppm
4. T_4- Coco peat + GA3 1500 ppm
5. T_5- Compost + GA3 0 ppm
6. T_6- Compost + GA3 1500 ppm
7. T_7- Rice bran + GA3 0 ppm
8. T_8- Rice bran + GA3 1500 ppm

2.3 Germination medium and Seed sowing

Flat germination trays of 60 cm x 30 cm were used. Each tray was divided into two halves to constitute two plots. As for the germination medium, easily available growth mediums like biochar, coco peat, compost, and rice bran were used. Seed sowing was done on 14th February in 2019 and 2020. Data recording was done on daily basis after the first germination on 18th March 2019 and 16th March 2020 till 4th May in both years. Irrigation was done on alternate days using a knapsack sprayer to avoid displacement of seeds from the media.

2.4 Data collection

Data were recorded in Microsoft Excel sheet. Advanced seed germination measurements excel tool developed by Dr. Frahan Khalid (Khalid, 2021) was used to calculate attributes of seed germination measurements like germination percentage, relativized percentage, mean germination time, time taken for 10% germination, and time taken for 25% germination.

2.4.1 Germination percentage

Germination percentage gives the total seed germinated out of the entire sample taken in an experiment (Labouriau & Viladares, 1976). It was calculated using the following formula:

$$G\% = \frac{\sum_{i=1}^{k} n_i}{N} \times 100$$

Where;

$n_i =$ number of seeds germinated in the i^{th} time

$N =$ total number of seeds used

2.4.2 Relativized percentage

It standardizes the assessment within the comparable treatments when there is a difference in the quantity of dormancy broken and it is calculated using the following formula (Fitch, Walck, Hidayati, & Ecology, 2007):

$$R(\%) = \frac{AP}{HP} \times 100$$

Where;

$AP =$ acutual percentage

$HP =$ highest percentage amongst group of data

2.4.3 Mean germination time

Mean germination time shows the time taken for the seed to emerge. The following formula was used to calculate it:

$$\bar{t} = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$$

Where;

$n_i t_i =$ The product of seeds germinated at the i^{th} time with the corresponding time interval

$n_i =$ number of seeds germinated in the i^{th} time

2.4.4 Time to 10% germination

Time to 10% germination gives the time taken for 10% of the seeds to germinate. T10 was calculated using the following formula:

$$T10 = \frac{t_i + (\frac{\sum_{i=1}^{k} n_i}{10} - n_i)(t_j - t_i)}{n_j - n_i}$$

To find out the value of n_i and n_j, we take the cumulative number of seeds germinated for which the condition is given below:

$$n_i < \frac{\sum_{i=1}^{k} n_i}{10} < n_j$$

Where;

$n_i =$ nearest cumulative number of seeds germinated $(Cn_i) < \frac{\sum_{i=1}^{k} n_i}{10}$

$n_J =$ nearest cumulative number of seeds germinated $(Cn_j) > \frac{\sum_{i=1}^{k} n_i}{10}$

$t_i =$ the time interval corresponding to n_i

$t_j =$ the time interval corresponding to n_j

We can calculate $T25$ by replacing $\frac{\sum_{i=1}^{k} n_i}{10}$ with $\frac{\sum_{i=1}^{k} n_i}{4}$

2.5 Data analysis

The Statistical Tool for Agricultural Research (STAR) 2.1.0 software was used to analyze pooled data for two years using Analysis of Variance (ANOVA) model. Data were tested for assumption for ANOVA, and data were found to be normal and variance homogenous, thus satisfying the assumption of ANOVA. Tukey's Honest Significant Difference (HSD) Test was

used for the Pairwise Mean Comparison of treatments since it is a robust method. Graphs and tables were constructed using MS Excel.

3. Results and Discussion

Effect of GA3 application and use of different substrates on kiwifruit seed germination are discussed in terms of seed germination measurement parameters like germination percentage, relativized percentage, mean germination time, time to 10% germination, and time to 25% germination.

3.1 Germination percentage

Actinidia species have exhibited high resistance to seed germination hence resulting in reduced seed emergence but the use of GA3 in Actinidia species was found to significantly ($P \leq .05$) affect germination percentage (Sekhukhune et al., 2016). Germination percentage was found to be considerably influenced by GA3 application and growing medium (Sharmaa et al., 2021). Our study found that the germination percentage was significantly affected by the use of various substrates and GA3 application (*P-value* < .004). Amidst the treatments, Coco peat + GA3 1500 ppm had the highest germination percentage of 51% followed by Biochar + GA3 1500 ppm, Coco peat + GA3 0 ppm and Biochar + GA3 0 ppm at 39%, 30% and 23% respectively. While the treatments with the lowest germination rates were rice bran (0 ppm and 1500 ppm) and compost (0 ppm and 1500 ppm) at 5% and 10% correspondingly. As per our study, the best germination percentage was acquired in treatments with cocopeat + GA3 1500 ppm as shown in Table 1. A similar study by Sekhukhune et al., (2016) found that GA3 treatment of 1435 to 1565 ppm was found to be preeminent for seed germination in kiwifruit. Likewise, cocopeat was found to considerably affect growth, germination, and development parameters due to its suitable physical, chemical, and biological properties (Bhardwaj, 2014). Biochar supplementation also offers a considerable influence on seed germination, shoot, and root growth (Bu, Xue, Wu, & Ma, 2020).

Table 1. Germination percentage of kiwifruit seeds under various treatments

Treatment	Mean
Biochar + GA3 0 ppm	23^{ab}
Biochar + GA3 1500 ppm	39^{ab}
Coco peat + GA3 0 ppm	30^{ab}
Coco peat + GA3 1500 ppm	51^{a}
Compost + GA3 0 ppm	10^{b}
Compost + GA3 1500 ppm	10^{b}
Rice bran + GA3 0 ppm	5^{b}
Rice bran + GA3 1500 ppm	5^{b}

P- value	< .004

Means with the same letter are not significantly different

GA3 treatment and medium combination had a paramount influence on seed germination, whereas a control treatment without GA3 treatment combined with a medium showed the least germination percentage (Celik et al., 2006).

3.2 Relativized percentage

The statistical value for relativized percentage of germination for various treatments was found to be significantly different (*P-value* < .004). The highest value was seen in T4 which is Cocopeat + GA3 1500 ppm followed by T2 (Biochar + GA3 1500 ppm) as given in Figure 1. As per Bhardwaj (2014) favourable physical and biological conditions in cocopeat have a good impact on germination and root development. Biochar provides good conditions for germination. Biochar is a conducive medium for earthworms (Raza et al., 2021). This could help in soil aeration and improve soil conditions.

The lowest value was observed in treatments with rice bran followed by compost. Low germination percentage in rice bran might be because of the presence of numerous antioxidant substances which cannot be decomposed easily (Nozoe et al., 2021). Whereas, compost performs better as a soil amendment. Supplementing the soil with compost enables the seed to germinate and establish itself in the soil (Paradelo et al., 2012) as compost acts as a fertilizer source by increasing soil nitrogen and decreasing C: N ratio (Raza et al., 2021).

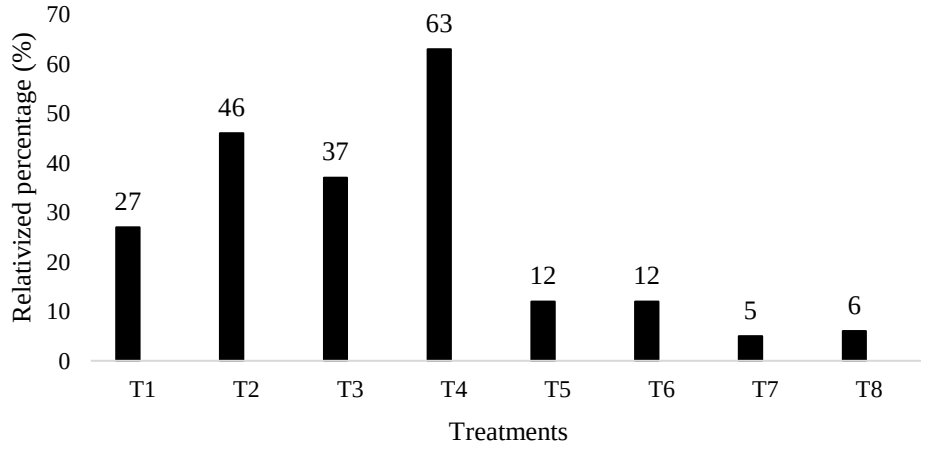

Figure 1. Relativized percentage for various treatments

3.3 Mean germination time

The overall germination time for our study was 50 days. The shortest germination time was observed in coco peat + GA3 0 ppm at 45 days and the longest germination time was taken in compost + GA3 0 ppm (Table 2). Varying germination time for each treatment was observed.

The combined effect of GA3 was significant, on top of it, different mediums influenced the whole germination time separately (Celik et al., 2006).

Table 2. Average time taken for seed germination

Treatment	Mean germination time (Days)
Biochar + GA3 0 ppm	55
Biochar + GA3 1500 ppm	51
Cocopeat + GA3 0 ppm	45
Cocopeat + GA3 1500 ppm	46
Compost + GA3 0 ppm	56
Compost + GA3 1500 ppm	47
Rice bran + GA3 0 ppm	53
Rice bran + GA3 1500 ppm	47
Grand mean	50

3.4 Time to 10% germination and Time to 25% germination

Overall, there is not much variation in the time taken for 10% germination and 25% germination for individual treatment. As for time taken to germinate 10% and 25% of seeds, T2 which is Biochar + GA3 1500 ppm took the minimum time (42 days and 44 days respectively) while T7 which is Rice bran + GA3 0 ppm took the maximum time (60 days and 61 days respectively) as shown in Figure 2.

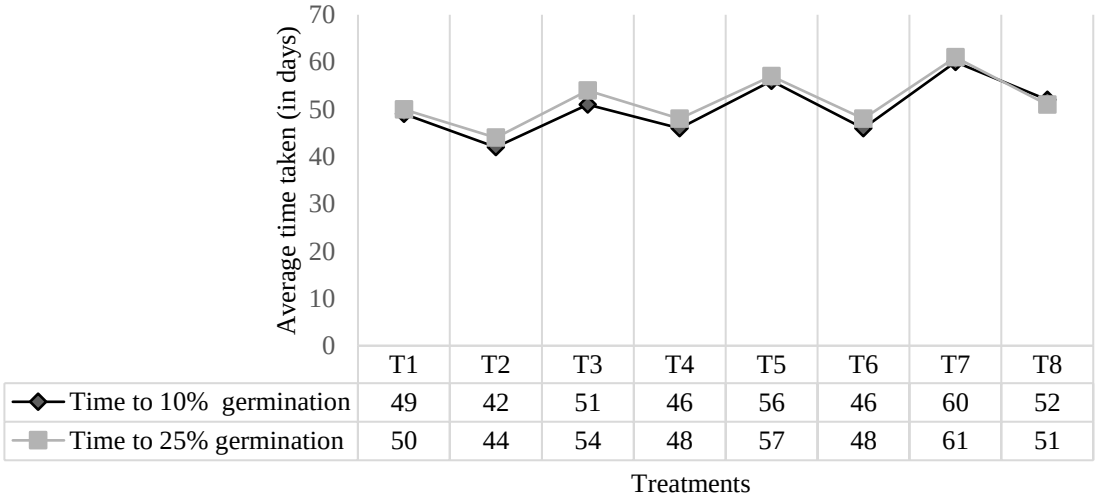

Figure 2. Time taken to germinate 10 and 25% seeds

GA3 treated seeds germinate faster and their mean germination time is lesser than water-soaked seeds (Adhikari, Dhital, Ranabhat, & Poudel, 2021). Similarly, in our study, control treatments without GA3 treatment and other treatments with GA3 treatment showed considerable differences in their germination time. GA3 treated treatments took comparatively less time to

acquire 10% and 25% germination. It can infer that the physiological dormancy of seeds can be prevented with GA treatment (Finch-Savage & Leubner-Metzger, 2006).

3.5 Overall ranking of growing media

To determine the most effective treatment combinations, the weighted average rank of mean germination percentage, germination time, relativized germination percentage, 10% and 25% germination percentage was calculated:

$$\text{WA} = \frac{\sum_{i=1}^{n} w_i X_i}{\sum_{i=1}^{n} w_i}$$

Where, WA is weighted average

w_i is weightage assigned to each parameter

X_i: list of parameters (germination percentage, germination time, relativized germination percentage, 10%, and 25% germination percentage)

Based on mean germination percentage, germination time, relativized percentage, 10% germination time, and 25% germination, the weighted average value for each treatment was estimated. Since the objective of the study is to study the effect of treatments on germination, the highest weightage was assigned to germination percentage, followed by relativized percentage, 10 and 25 percentage and time of germination. Overall, Cocopeat + GA3 1500 ppm received the highest weighted average score and rank as number one making it the most suitable treatment to enhance kiwifruit seeds germination followed by Biochar + GA3 1500 ppm and Cocopeat + GA3 0 ppm (Table 3).

Table 3. Overall ranking of weighted average

Treatments	Weighted average rank
Coco peat + GA3 1500 ppm	1
Biochar + GA3 1500 ppm	2
Coco peat + GA3 0 ppm	3
Biochar + GA3 0 ppm	4
Compost + GA3 0 ppm	5
Compost + GA3 1500 ppm	5
Rice bran + GA3 1500 ppm	7
Rice bran + GA3 0 ppm	8

4. Conclusion

In conclusion, the presented result showed that the combined effect of GA3 and different growth mediums can significantly enhance germination percentage in kiwifruit seeds. Based on the final weighted average of germination parameters, we can deduce that the use of growing mediums like cocopeat and biochar along with 1500 ppm GA3 seed treatment can help in obtaining satisfactory seed germination.

The combined effect of GA3 and the use of suitable mediums like cocopeat and biochar gave the highest germination percentage of 51 % and 39 % respectively. GA3 treatment also helped in accelerating mean seed germination time. GA3 treatment in combination with all the germination mediums reduced the time taken to achieve 10% and 25% germination to an average of 47 and 48 days, respectively.

In our study, we observed that cocopeat enhanced seedling emergence but it remained stunted throughout the study period while emerged seedlings in biochar medium grew to a considerable height. Therefore, we would also like to recommend further study to assess the parameters of kiwifruit growth and development on top of seed germination through the combined use of germination medium and GA3 application.

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