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Interactive Effect of Growth Medium, Moist Chill Stratification and Gibberellic Acid Scarification on Kiwifruit (*Actinidia deliciosa*) Seed Germination

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Abstract

Kiwifruit is an economically important temperate fruit crop propagated commercially through grafting. Seed germination is essential for the production of vigorous rootstocks; however, physiological dormancy often limits germination performance. The present study evaluated the interactive effect of growth medium, moist chill stratification, and gibberellic acid (GA_3) application on kiwifruit seed germination under greenhouse conditions at Wengkhar Agriculture Research and Development Centre, Bhutan. A 4×2×2 factorial experiments arranged in a Completely Randomized Design with three replications was used. Four growth media (cocopeat, biochar, rice bran, and compost), two stratification durations (24 hours/0-week and eight weeks), and two GA_3 concentrations (0 and 1500 ppm) were evaluated.

Among the growth media, cocopeat treated with 1500 ppm GA_3 recorded the highest germination percentage (52.83%), whereas rice bran exhibited the lowest germination performance. The mean germination time averaged 50 days, the coefficient of variation of germination time averaged 71.88%, and the mean germination rate averaged 0.01 day-^{1}, with neither parameter significantly affected by growth medium, stratification, or gibberellic acid treatment. Moist chill stratification showed no significant effect on germination percentage, suggesting that maintaining seed moisture before sowing effectively reduced dormancy. The interaction between growth medium and GA_3 significantly improved germination performance. The study demonstrates that cocopeat combined with 1500 ppm GA_3 is suitable for enhancing kiwifruit seed germination and rootstock production.

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Introduction

Kiwifruit *(Actinidia deliciosa)* is a deciduous fruit adapted to wide climatic range, with unique blend of taste and high nutritive and medicinal values. Kiwifruit cultivation originated in China and was later commercialized in New Zealand, which remains a leading producer along with China, Italy, Iran, and Chile; major cultivated varieties include the green-fleshed Actinidia deliciosa and the gold-fleshed Actinidia chinensis. In Bhutan, kiwifruit is cultivated mainly in the mid-hills at elevations between 1000 and 2500 m, and has been promoted as a high-value crop suited to sloping land, with potential to diversify farm income and improve soil conservation in fruit-growing dzongkhags. Kiwifruit is gaining popularity in the fresh market due to its high returns per unit area. The varieties Wengkhar Green, Yellow Joy, Enza Red, and Hayward are commonly grown for their adaptability and fruit quality. In eastern Bhutan, Wengkhar Green and Yellow Joy are particularly popular, following their promotion by the Agriculture Research and Development Center, Wengkhar.

Kiwifruit is typically propagated through grafting in commercial cultivation as vegetative cuttings exhibit poor rooting ability. The increased demand of seedlings could not be met through cuttings, the mass multiplication of rootstocks through seeds was essential. The rootstock raised from seeds are more vigorous and enhance adaptation ability due to stronger root system than plants propagated from cuttings (Celik *et al.*, 2006; Ahmad, 2010).

Despite its importance, kiwifruit seed germination is constrained by physiological dormancy and seed recalcitrance, resulting in poor and uneven emergence. Various dormancy-breaking techniques, including cold stratification, scarification, and gibberellic acid (GA_3) application have been employed to improve germination performance (Hartmann *et al.*, 2001). Many studies used cold stratification and gibberellic acid which showed varying germination percentage of kiwi and other hardy fruits while there was comparative interaction effect of cold stratification and GA_3. The germination rate was 20% when cold stratified for 4 weeks and no germination at 6 weeks. The germination percentage was 91% and 48% when treated with 1572 ppm and 2225 ppm GA_3 but without cold stratification. However, germination rate increased to 88% when cold stratified for 4 weeks was treated with 1610 ppm GA_3 and germination rate was increased to 99% when 6 weeks cold stratified seeds were treated with 1563 ppm GA_3 (Sekhukhune *et al.*, 2016). Similarly when the kiwi seeds were cold stratified at 4.4ºC for 6 weeks, the germination percent was 53% while the germination percent was 67% when cold stratified seeds were treated with 1500 ppm GA_3 (Ahmad, 2010). The increase in application of GA_3 from 0 ppm to 6000 ppm did not affect the germination rate but with peat moss medium the germination of 78% was achieved even at 0 ppm GA_3 which indicated

that constant moisture need to be ensured (Celik, Zenginbal, and Özcan, 2006). Several studies conducted on the germination of kiwi indicated that the minimum of 4 weeks of cold stratification and 1500 ppm GA_3 application was required to enhance the germination percentage. In the previous studies, seeds were dried, packed in plastic pouch and stored in the refrigerator which may have induced seed dormancy. The moist chill stratification ensures constant moisture during seed storage and prevents seed entering into dormancy, the seeds can be sown immediately after extraction. Previous studies have reported variable germination responses depending on the duration of cold stratification and GA_3 concentration. However, limited information is available on the combined interactions among growth media, moist chill stratification, and GA_3 application under Bhutanese conditions. Therefore, this study evaluated the interactive effects of different growth media, moist chill stratification durations, and gibberellic acid concentrations on the germination performance of kiwifruit seeds.

Materials and Methods

Study area and Experimental design

The experiment was conducted from September to October, 2024 under greenhouse conditions at Wengkhar Agriculture Research and Development Centre, Mongar, Bhutan. During the study period, the average greenhouse temperature ranged approximately between 25-30°C with relatively high humidity conditions favourable for seed germination. The study consisted of 4 × 2 × 2 factorial experiments arranged in a Completely Randomized Design (CRD) with three replications. The experimental factors included four growth media, two moist chill stratification durations, and two levels of gibberellic acid application. Each treatment consisted of 100 seeds sown in germination trays measuring 30 × 15 cm.

The experiment was carried under greenhouse condition, where the greenhouse temperature ranged from 25°C to 30°C and relative humidity ranged conditions favourable for seed germination. The study used 4x2x2 factorial in Completely Randomized Design with 16 treatments and three replications. The main factor comprised of four growth mediums, two stratification duration and two Gibberellic acid applications.

Plant material and growth media

The experiments were carried out on four growth medium Coco peat, Biochar, Rice bran and Compost, based on the water holding capacity and its easy availability. Seeds were extracted from the Wengkhar green variety of kiwifruit. In each treatment, 100 kiwifruit seeds were used as a unit of study. The seeds were sown in germination trays with 30x15 cm dimension.

Moist chill stratification

In the moist chill stratification, the seeds were extracted from ripe, uniformly sized green kiwifruits. The seeds were manually separated from the pulp, thoroughly

washed, and mixed with moist sand before being placed in plastic containers. The containers were then stored in a refrigerator at a constant temperature of 4.5°C to provide moist chilling stratification while maintaining adequate seed moisture. Two stratification durations were evaluated: 24 hours and 8 weeks. The 24-hour treatment was designated as the 0-week stratification treatment because the seeds were maintained under moist conditions for only 24 hours and were not subjected to extended cold stratification before sowing.

Gibberellic acid scarification

In this treatment, the seeds were soaked in 0 ppm (distil water) and 1500 ppm of gibberellic acid for 12 hours, under ambient room-temperature conditions.

Germination parameters

Seven germination parameters were collected, calculated, and reported throughout the experiment according to the procedures outlined by (Ranal *et al.*, 2009). The parameters are Germination Percentage (G); Mean Germination Time (MT); Coefficient of Variation of Germination Time (CVt); Mean Germination Rate (MR); Uncertainty of Germination Process (U); Synchrony of Germination Process (Z); and Coefficient Velocity of Germination (CVG).

The germination data were collected from the day of first germination and stopped when there was no germination for three consecutive days. The irrigation was provided every alternate day.

Statistical analysis

A factorial analysis of variance was used to analyze the main effect and interaction between growth medium, moist chill stratification and GA_3 application. The significant mean difference was separated using Tukey's Honest Significant Difference post hoc test. All the analysis was based on 95% confidence level using Star software.

Result and Discussions

This study evaluated the interaction effect of growth medium, stratification and GA_3 application on the germination percentage of kiwi seeds. The analysis showed both main effect and interaction effect of the three factors on all the germination parameters. In terms of main effect, growth medium showed significant effect followed by GA_3 scarification, however the moist chill stratification had no significant effect on germination performance.

Germination Percentage

There was a significant difference in germination percentage with different growth medium. The germination percentage (53% and 52%) was significantly higher in cocopeat medium in seeds treated with 1500 ppm GA_3 at 0 week and 8 weeks of

stratification. At 0 ppm GA_3 the germination percentage was only (29% and 40%) respectively at both moist chill stratifications. The lowest germination percent was observed in rice bran (2%) with no significant difference in moist chill stratification and GA_3 scarification. The higher germination of the kiwi seeds in cocopeat is due to higher moisture retention capacity. The irrigation was provided every alternate day, although the water retention capacity was not measured, through visual inspection the retention capacity was higher in cocopeat. The water retentive capacity of compost is low as compared to other mediums. The rice bran tends to form a hard layer when dried limiting the water to percolate during irrigation. The availability of moisture in coco peat with desired temperature induced the higher germination percentage. The further research could be done to assess the mixture of growth medium for higher germination and seedling vigor under different temperature regime. There was a significant difference in germination percentage with different GA_3 application. Similar findings was reported by (Zhang *et al.*, 2018; Bishwas *et al.*, 2018) where the increase in GA_3 concentration promoted higher germination percentage and 1500 ppm GA_3 is optimum dosage for enhanced germination (Ahmad, 2010).

Germination Time, Rate and Synchrony

The average germination time was 50 days indicating no significant interaction effect on the mean germination time. Similarly, there was no significant effect on the germination rate with average rate of 0.01 day^{-1}. There was no significant difference in synchrony of germination indicating no overlapping in germination. The overall mean of Z is 0.09 indicating there was less numbers of seeds germinating at a same time or negligible degree of overlapping. This study corroborates with Bishwas *et al.* (2018) in terms of no significance difference in the synchrony of germination in kiwi seeds.

Uncertainty of Germination Process

The cocopeat at 1500 ppm GA_3 showed higher uncertainty (4.36 bit) at 8 weeks stratification and (4.34 bit) at 0-week stratification which is not significantly different. The degree of spread of germination time (0.78 bit) was least for compost at 0 week and 1500 ppm.

Coefficient of Velocity of Germination

The growth medium and interaction effect with cold stratification showed significant difference in the mean velocity of germination. The germination per unit time was higher in biochar (2.5%) treated with 1500 ppm GA_3. The lowest germination velocity was in rice bran (1.3%) both the cold stratification level.

Table 1. Effect of Growth Medium, Stratification and Gibberellic Acid Concentration on the germination of kiwifruit seeds

Medium	Stratification	GA_3	G	MT	CVt	MR	U	Z	CVG
Biochar	0 week	0 ppm	22.83^{ab}	49.17	51.84	0.02	1.99^{ab}	0.09	2.07^{ab}
Biochar	0 week	1500 ppm	38.67^{ab}	41.47	81.43	0.02	3.14^{ab}	0.11	2.5^{a}
Biochar	8 weeks	0 ppm	16.67^{ab}	50.50	92.34	0.02	1.23^{ab}	0.1	2.05^{ab}
Biochar	8 weeks	1500 ppm	10.67^{b}	49.88	91.00	0.02	1.64^{ab}	0.04	2.02^{ab}
Cocopeat	0 week	0 ppm	29.17^{ab}	52.96	45.67	0.01	2.65^{ab}	0.06	1.94^{ab}
Cocopeat	0 week	1500 ppm	51.50^{a}	46.93	39.65	0.02	4.34^{a}	0.07	2.18^{ab}
Cocopeat	8 weeks	0 ppm	39.67^{ab}	49.16	65.89	0.02	3.72^{ab}	0.04	2.1^{ab}
Cocopeat	8 weeks	1500 ppm	52.83^{a}	49.31	87.11	0.02	4.36^{a}	0.07	2.16^{ab}
Compost	0 week	0 ppm	10.17^{b}	50.91	84.49	0.01	1.46^{ab}	0.02	1.43^{b}
Compost	0 week	1500 ppm	15.83^{ab}	62.93	64.81	0.01	0.78^{b}	0.03	1.62^{ab}
Compost	8 weeks	0 ppm	18.67^{ab}	61.65	41.27	0.01	2.04^{ab}	0.21	1.67^{ab}
Compost	8 weeks	1500 ppm	21.00^{ab}	54.62	108.7	0.01	3.18^{ab}	0.19	1.86^{ab}
Rice bran	0 week	0 ppm	4.83^{b}	57.44	79.25	0.01	1.09^{ab}	0.07	1.75^{ab}
Rice bran	0 week	1500 ppm	4.50^{b}	54.99	91.71	0.01	0.83^{b}	0.24	1.85^{ab}
Rice bran	8 weeks	0 ppm	2.00^{b}	38.21	69.55	0.01	0.83^{ab}	0.05	1.18^{b}
Rice bran	8 weeks	1500 ppm	14.33^{ab}	34.66	55.40	0.01	1.8^{ab}	0.08	1.3^{b}
	Significance		**	ns	ns	ns	**	ns	**
	p-value		0	0.09	0.11	0.01	0.002	0.68	0.001
	Grand mean		22.08	50.3	71.88	0.01	1.99	0.09	1.85
	Tukey's HSD		39.67				4.77		1.04

Conclusion

The present investigation demonstrated the significant impact of the growth medium on the germination process of kiwifruit seeds. Furthermore, the introduction of 1500 ppm of gibberellic acid remarkably augments germination rates. Results indicate that maintaining kiwifruit seeds under moist chilled conditions effectively mitigates dormancy, facilitating convenient sowing during the growth season. While direct sowing of seeds post-extraction from fruits is feasible, prolonged storage raises concerns regarding potential fruit infection or damage. Notably, this study omits an evaluation of seedling vigor, hypothesizing its correlation with the limited nutrient availability in the growth medium. To address this, future endeavors may necessitate additional nutrient supplementation or the adoption of mixed growth media to strengthen seedlings until they attain transplanting maturity.

The present study demonstrated that growth medium and gibberellic acid application significantly influenced kiwifruit seed germination. Cocopeat combined with 1500 ppm GA_3 produced the highest germination percentage and improved germination performance. Moist chill stratification effectively maintained seed viability and minimized dormancy during storage. The findings suggest that the use of cocopeat together with GA_3 treatment can be recommended for efficient kiwifruit rootstock production under Bhutanese conditions. Future studies should evaluate seedling vigor and nutrient supplementation strategies for improving transplant establishment.

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