



Sowing Media and Gibberellic Acid Influences Germination and Seedling Growth of Chilgoza Pine (*Pinus gerardiana*. Wall)

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Authors' contributions

This work was carried out in collaboration among all authors. Author HS designed the study and wrote the protocol. Author SSK and OAW managed the analyses of the study, performed the statistical analysis, wrote the first draft of the manuscript and performed critical revision of the manuscript. Author SK and VVK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Pinus gerardiana Wall. recognized for its significant contribution to nut production, holds ecological and economic importance as a forestry species with limited range primarily found in Afghanistan. The species displays either extremely low or non-existent levels of natural regeneration. Due to

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irregular and uncommon seed years, problems with dormancy, and other factors, this species' natural habitat renewal is slowed down. Hence, we conducted a study to examine how different substrates and gibberellic acid (GA₃) affect the germination and growth of Chilgoza pine seedlings. The substrates were grouped into three types, each containing varying proportions of soil, sand, and farmyard manure (FYM): M₁ (1:1:1), M₂ (1:1/2:1/2), M₃ (1:1/3:2/3). Additionally, three concentrations of GA₃ (0, 50, and 100 ppm) were used to assess their impact on germination and seedling growth. The results revealed that, with the exception of mean germination time (MGT) at 42.36 days, the highest values for final germination percentage (FGP) at 58.66%, germination index (GI) at 274, germination rate index (GRI) at 75.01, shoot fresh weight (SFW) at 8.74 g, shoot dry weight (SDW) at 4.78 g, shoot length (SL) at 219.80 mm, and seedling vigor index (SVI) at 1291926.02 were achieved when seeds were initially soaked in a 100 ppm GA₃ solution for 6 hours and then planted in a medium with equal proportions of soil, sand, and FYM. The effects of GA₃ concentrations were significant in influencing seedling growth, while substrate effects strongly impacted seed germination. This indicates that Chilgoza seeds possess ample gibberellin for germination but this is reduced during the seedling growth phase. The response of seedling vegetative growth to GA₃ concentrations exhibited a notable linear trend, whereas the relationship between FGP and MGT with GA₃ concentrations showed a significant quadratic pattern. This suggests that GA₃ concentrations can effectively promote seedling growth. The substrate choice notably influenced FGP and MGT due to its role in nutrient provision and aeration. The highest GA₃ concentration led to the greatest enhancement in seedling vegetative growth, implying that prolonged usage might yield further benefits over time. In conclusion, the findings suggest that both GA₃ and substrate have the potential to enhance Chilgoza seed germination and subsequent seedling growth, and their combined application could be a beneficial approach.

Keywords: Ecological; gibberellic acid; *Pinus gerardiana* wall; regeneration; substrate.

1. INTRODUCTION

Chilgoza pine (*Pinus gerardiana* Wall.) stands as a remarkable ecological and commercial tree species, gracing the landscapes of Afghanistan, Pakistan, and the temperate Himalayan region of India [1]. Its intrinsic ecological significance, coupled with its high economic importance, makes it a valuable asset to the region, particularly for Afghanistan. Known by various names such as "Chilgoza" or "neoza pine," this native forest species belongs to the esteemed *Pinaceae* family and was first encountered in India in 1932 by Captain Gerard, a British officer [2]. The Chilgoza pine showcases a graceful perpetual appearance, manifesting as a tree of modest to intermediate proportions, reaching heights spanning 17 to 27 meters and possessing girths that measure 2 to 4 meters [3]. Within the *Pinus* genus, *Pinus gerardiana* stands out as a species of profound significance. It yields edible nuts recognized for their substantial nutritional and economic worth, commonly referred to as Chilgoza nuts, sought after both domestically and internationally [4]. *Pinus gerardiana* Wall. is not only a species of ecological and commercial significance but also holds cultural and traditional value for the communities residing in the regions where it flourishes. The Chilgoza pine forests have been

an integral part of the local way of life for generations, providing a source of livelihood and sustenance to the indigenous populations [5].

The edible Chilgoza nuts, produced abundantly by the *Pinus gerardiana*, have been a staple food and an essential component of the local cuisine for centuries. These nutrient-rich nuts are not only delicious but also highly nutritious, containing essential fatty acids, proteins, and a variety of vitamins and minerals [6]. As a result, Chilgoza nuts have become a sought-after delicacy in both local and international markets, contributing significantly to the economies of the regions where they are harvested. Chilgoza holds a notable position in contributing to the socio-economic advancement of rural communities in Afghanistan. The native environment of *Pinus gerardiana* in Afghanistan, India, and Pakistan primarily comprises fully grown and over-mature trees, whereas the presence of seedlings and young trees is confined to extremely challenging terrains and regions enveloped by prickly shrubbery. According to international figures from 2014, China and Afghanistan were the world's top and third producers, contributing 62% and 8% of the world's production with a yearly output of 25000 and 3100 metric tons, respectively [7]. Since 2018, the trade value of Afghanistan Chilgoza

has improved due to an agreement between China and Afghanistan. Nonetheless, excessive harvesting and the presence of grazing livestock (such as sheep and goats), coupled with intrinsic seed dormancy, stand out as significant issues that contribute to the limited regeneration of the species. These challenges have led to the classification of the species within the endangered category as recognized by the International Union for the Conservation of Nature [IUCN] [8]. The increasing demand for Chilgoza nuts has put substantial pressure on the natural Chilgoza pine forests. Unsustainable harvesting practices and deforestation have led to concerns about the long-term sustainability of these valuable ecosystems. Conservation efforts and sustainable management practices have become imperative to safeguard the future of Chilgoza pine and its associated biodiversity.

Natural regeneration has been observed as either completely absent or very low in the species. The primary cause of this is the gathering of cones by the local population [9]. This important species has vanished or been exterminated due to severe biotic interference and a lack of regeneration [10]. Given the critical importance of Chilgoza pine in the ecological balance and local economies, urgent action is required to prevent its extinction. The key to preserving this species lies in developing effective mechanisms for regeneration. Central to this effort is enhancing the germination process, which is crucial for improving the overall regeneration of a species. Higher germination rates and robust seedling growth are vital for successful forest conservation and restoration initiatives [11]. Researchers, conservationists, and local communities are joining forces to implement measures that promote Chilgoza pine regeneration [12]. Protective measures are being introduced to reduce the gathering of cones from mature trees, allowing them to contribute to seed dispersal naturally [13]. Community-based awareness programs are educating the local population about the importance of sustainable harvesting practices and the significance of safeguarding the species for future generations. In addition to addressing the human-induced challenges, research is focused on understanding the germination requirements and optimal conditions for Chilgoza pine seedlings [14].

Growth medium significantly impacts seed germination, growth, and nursery seedling quality [15]. Quality planting material is crucial for the

successful development of a plantation since the establishment of plants depends on their ability to tolerate challenging environmental circumstances. Seedling establishment heavily relies on the critical stages of germination, which can be significantly hindered when exposed to drought stress [16]. The germination process, as well as its surrounding processes, can be negatively affected by variations in the water content of the growth medium, including both excess and scarcity [17]. Earlier research has tackled this matter by exploring diverse osmotic potentials of moist substrates utilizing polyethylene glycol (PEG6000) solutions, effectively mimicking conditions of water scarcity for seeds [18, 19]. This widely adopted approach has been utilized across various commercial seed species to analyze their reactions to water stress. The composition of the growth medium plays a pivotal role in determining the quality of the planting material, subject to alteration by other variables. A study conducted by Sappalani et al. [20] demonstrated the significant influence of both soil media and GA₃ on the germination and seedling growth of distinct forest tree seeds. Additionally, Bhardwaj et al. [21] established that a soil medium containing an equal blend of soil, sand, and FYM yielded optimal conditions for the germination and seedling growth of *Pinus roxburghii*. Several investigations have unveiled noteworthy impacts of GA₃ on seed germination. For instance, Amri's study [22] revealed the concentration-dependent effect of GA₃ on the seed germination of *Terminalia sericea*. Similarly, Kumar et al. [10] identified a positive correlation between GA₃ application and the germination of *Pinus gerardiana* seeds.

Chilgoza pine is characterized by a notably sluggish growth rate, requiring approximately 3 to 4 years attaining a size suitable for transplantation [23]. This predicament underscores the immediate necessity for a study aimed at expediting the process of achieving an appropriate size for planting and the creation of premium-quality nursery planting materials. The interaction between the substrate and GA₃ might yield significant effects on both the germination and initial growth of *Pinus gerardiana* seedlings. However, despite this potential interplay, no prior endeavor has been undertaken to evaluate their combined impact on Chilgoza pine. Hence, the present investigation was carried out with the intent of elucidating the influence of substrates and varied GA₃ concentrations on the germination and growth dynamics of Chilgoza pine seedlings. The present findings from our

research can offer a game-changing advantage for Chilgoza nursery growers, empowering them to unlock the seeds' full germinating potential and propel their nursery production cycle to new heights. The benefits of this innovation extend not only to nursery growers but also to the ecological balance and economic well-being of the regions where Chilgoza pine thrives. This may not only accelerate the growth of Chilgoza pine seedlings but also ensures sustainable practices and conservation efforts for the preservation of this slow-growing species and its ecosystems. This study embraces and embarks on a transformative journey toward enhanced Chilgoza pine nursery production, ecological preservation, and economic prosperity.

2. MATERIALS AND METHODS

2.1 Study Site

Paktia province is located in eastern Afghanistan and is situated between approximately 33.6° N latitude and 69.5° E longitude (Fig. 1). Paktia is characterized by its predominantly rugged terrain, with a majority of its population residing within the central valley that extends from Ahmadkhel in the eastern part and stretches through Zurmat, reaching into the neighboring province of Paktika. Notably, the valleys of Tsamkani and Dand Aw Patan carve their way through the eastern region of the province, extending towards Pakistan. Paktia, an Afghan province in the southeast, only makes up 0.90% of the country's total land area, with a total size of 5583.20 km². Approximately 590668 individuals are living there, 301873 men and 288795 women. This province's population density is predicted to be 106 people per square kilometer. The region experiences a cold, semi-arid Mediterranean environment with significant winter snowfall. The summertime climate is quite warm, with temperatures reaching as high as 35°C; the wintertime environment is chilly, with low temperatures ranging from -10 to -20°C. It is among one of the provinces in the country's southeast region bestowed with dense natural forest cover. The region has multiple land-use systems ranging from open and closed forests, native grasslands, diverse agroforestry, and water bodies. Despite such systems, wastelands are greater in the area, followed by forest scrubs, open forests, and other land-use types. Only around 40% of the ecoregion is vegetated, mostly in the form of open woodland, bushes, and herbaceous cover. Altitude zones mostly determine the type of forest. The forest

composition within the specified elevation range showcases distinct species such as the Chilgoza pine (*Pinus gerardiana*), holly oak (*Quercus baloot*), various plants belonging to the beech family (*Fagaceae*), and cedar. This woodland area, situated between 2,100 and 2,500 meters above sea level, is characterized by relatively drier conditions, particularly evident in the cedar (*Cedrus*) species. As one ascends to higher altitudes, ranging from 2,500 to 3,100 meters, the influence of monsoon rains becomes more pronounced. Within this range, a transition occurs, and a mix of deciduous trees emerges alongside conifers. Notable inhabitants of this zone include the Morinda spruce (*Picea smithiana*), Bhutan pine (*Pinus wallichiana*), *Quercus semecarpifolia*, and the Himalayan cedar (*Cedrus deodara*). This potential dense woodland at higher elevations demonstrates a diverse array of tree species, contributing to the intricate and dynamic ecosystem. The woodland changes to more juniper at elevations above 3,100 meters (*Juniperus seravschanica*). Wood, lumber, and gas make up most of this province's natural resources. Rice, potatoes, maize, and wheat are all crops grown by farmers. The province is home to pomegranate, grape, peach, almond, and apple orchards. To provide milk, meat, and eggs for household consumption, as well as the market and transportation, farmers raise milking cows, sheep, goats, donkeys, and chickens. In the valley areas, alluvial subsoils with loess top layers are typical. These soils are calcareous and contain a lot of calcium carbonate (CaCO₃). Soil pH is often high, ranging from 8.00 to 8.50. Tillage and nutrients are well-tolerated by these soils.

The present study conducted in 2018 spanned from March to August, taking place at the Agriculture Research Farm of Paktia University, situated at coordinates 33°38'53" N and 69°01'58" E in Gardiz city (Fig. 1). Employing a completely randomized design (CRD), the experiment featured two factors, three replications per treatment, and 50 seeds per replication, all centered to the Chilgoza pine (*Pinus gerardiana* Wall.) seeds. The seeds were procured from local individuals residing near the Chilgoza forest within Paktia province. One-gram packets of GA₃ were sourced from the local market, available in powdered form. To create various GA₃ concentrations (control - GA₀ [0 ppm], GA₁ [50 ppm], and GA₂ [100 ppm]), an initial amount was dissolved in 1 ml⁻¹ ethanol. Subsequently, distilled water was added to attain

the desired concentrations. Regarding the soil substrate, distinct volume ratios of soil, sand, and farmyard manure (FYM) were employed: M₁ (1:1:1), M₂ (1:1/2:1/2), and M₃ (1:1/3:2/3). The procedure began with treating the seeds for a six-hour duration with diverse GA₃ concentrations, allowing them to subsequently air-dry. Following this, the seeds were promptly submerged in a diluted solution of TERM fungicide (0.5ml L⁻¹). These treated seeds were then sowed in polybags containing different substrates, after which they were placed within a greenhouse environment. Throughout the study, manual irrigation was administered on a weekly basis using a water sprayer, and weeding was carried out by hand as needed.

The time taken for the initial germination event was noted, and subsequently, the count of germinating seeds was documented daily. The 28th and 68th days were respectively marked as the first and final germination days, demarcating the periods before and after which no further germination occurred. To gauge weight metrics, seedlings were delicately uprooted from the

polybags. Initially, the length of the seedlings was measured utilizing a standard ruler, spanning from the crown to the highest point. Subsequently, the seedlings were weighed to determine their fresh weight, using a digital scale. Following this, the seedlings were transferred to an oven and subjected to a temperature of 70°C for a duration of 72 hours. Upon completion of the drying process, the dry weight of the seedlings was measured. Certain parameters were computed using the subsequent formulas:

2.2 Final Germination Percentage (FGP)

The final germination percentage was computed according to Kumar [24] using the formula;

$$FGP = \frac{\sum GS}{\sum SS} \times 100 \quad (1)$$

Where, $\sum GS$ = sum of the number of seeds germinated from 28th to 68th days after sowing and $\sum SS$ = the total number of seeds sown in replicate (50 seeds).

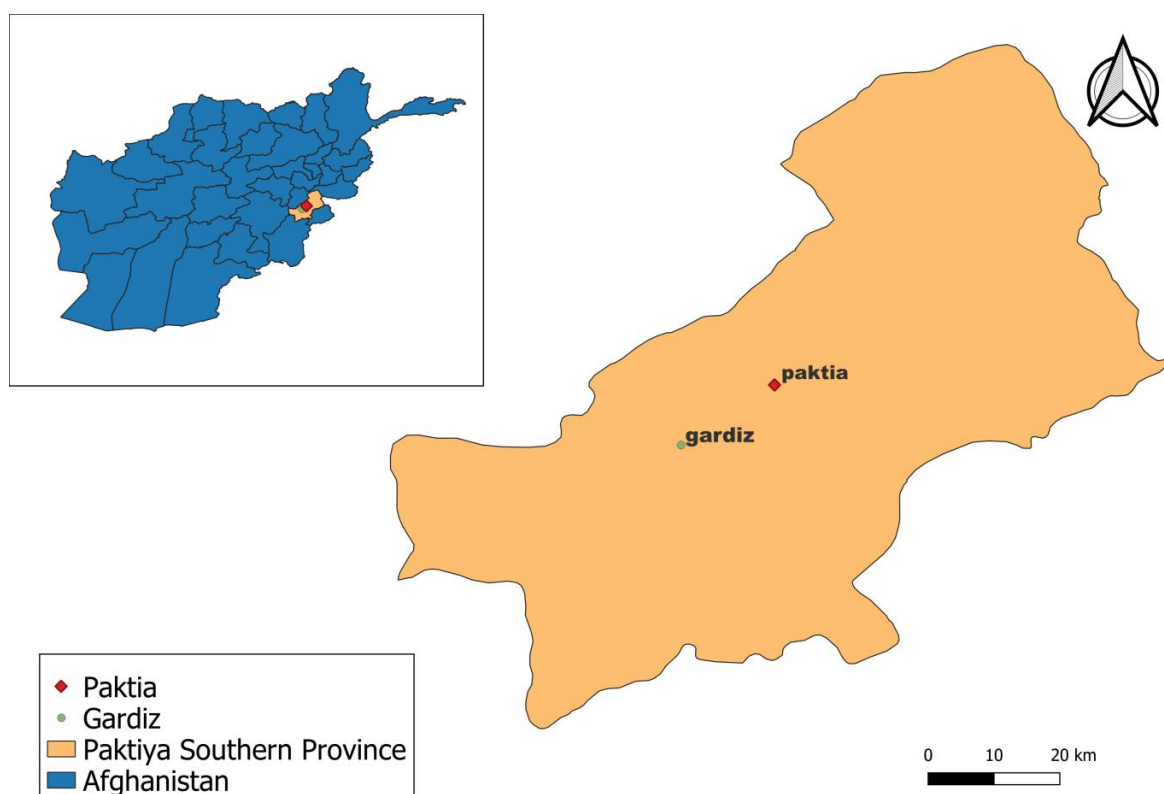


Fig. 1. Map of the study area

2.3 Mean Germination Time (MGT)

The mean germination time was computed according to Maguire [25] using the formula;

$$MGT = \frac{\sum F \cdot X}{\sum F} \quad (2)$$

Where,

$\sum F$ = number of germinated seeds
 X = number of days taken for germination of F seeds.

2.4 Germination Index (GI)

The germination index was calculated according to Bewley et al. [26] using the formula;

$$GI = (10 \times N1) + (9 \times N2) + \dots + (1 \times N10) \quad (3)$$

Where, N1, N2, N3.... N10 shows the numbers of seeds germinated in the 1st, 2th, 3th...10th days after cultivation.

2.5 Germination Rate Index (GRI)

The germination rate index was calculated according to Maguire [25] using the formula;

$$GRI (\%/day) = \frac{P_1}{1} + \frac{P_2}{2} \dots + \frac{P_x}{x} \quad (4)$$

Where P₁= percentage of seeds germinated on the 1st day, P₂= percentage of seeds germinated on the 2nd day, P₃ is the percentage of germination on the 3rd day, and x= number of days after seed sowing.

2.6 Seedling Vigor Index (SVI)

The seedling vigor index was computed according to Abdul-Baki and Anderson [27] using the formula;

$$SVI = SDW \times FGP \quad (5)$$

Where,

SDW= seedling dry weight
 FGP= final germination parentage.

2.7 Analysis

Data were processed and analyzed for Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using SAS® (Version#9) to test significance at 5% and mean comparisons.

3. RESULTS

The combination of substrate and GA₃ significantly impacted the FGP, as indicated by the results in Table 1. Specifically, treatments M₁ and GA₂ yielded FGP rates of 55.33% and 51.55% respectively, while the combined treatment of M₁ and GA₂ resulted in an FGP of 58.66% Table 1. The LSD test demonstrated notable statistically significant distinctions both within and between the effects of substrate, GA₃, and their interaction, as illustrated in Fig. 2. Upon closer examination through simple effects analysis, it was evident that the highest GA₃ concentration (100 ppm) was not as effective as the soil substrate (M₁) in promoting germination. However, the interaction between GA₃ and substrate showed potential for enhancing germination Table 1. The data in Table 1 further illustrates that as GA₃ treatment concentrations gradually increased from 0 ppm to 100 ppm, the germination percentage exhibited a gradual increment from 40.22% to 51.55%, representing an approximate 22% rise. The observed response trend of FGP in relation to GA₃ concentrations demonstrated a notably positive linear and quadratic pattern.

The analysis of data revealed that both substrate and GA₃ treatments had a notable impact on MGT, as detailed in Table 2. Conversely, the interaction between substrate and GA₃ did not demonstrate a significant influence on MGT. Among the substrate treatments, M₁ displayed the shortest MGT at 42.36 days, while M₂ exhibited the longest MGT at 45.25 days, indicating a reduction of 6.5% in MGT. The disparity between M₁ and M₂ was statistically significant, as depicted in Fig. 3. In terms of GA₃ treatments, the briefest MGT (42.38 days) was observed with GA₁ (50 ppm), while the longest MGT (45.16 days) was associated with GA₂ (100 ppm), resulting in a comparable 6.5% decrease Table 2. Notably, significant differences were observed between GA₁ and GA₂ treatments, while no statistically significant difference was observed between GA₀ (0 ppm) and GA₂ (100 ppm) Table 2. Furthermore, the relationship between MGT and GA₃ concentrations exhibited a significant quadratic pattern, implying a non-linear response to varying GA₃ concentrations.

The analysis of variance revealed a significant interaction effect between the substrate and GA₃ on GI, as depicted in Fig. 4. The greatest GI value (250.889) was achieved from seeds

cultivated in M₁ soil substrate, while the lowest GI (102.88) was observed in seeds grown in M₂ soil substrate. This difference between M₁ and M₂ was statistically significant, as indicated in

Table 3. Notably, when seeds were pre-treated with 100 ppm GA₃ (GA₂) and subsequently sown in M₁ soil substrate, the highest GI (274) was attained. Conversely, seeds sown in M₃ without

Table 1. Final germination percentage as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	57.33 ^{ab}	50.00 ^c	58.67 ^a	55.33 ^a
M ₂	44.00 ^d	43.33 ^d	52.00 ^{bc}	46.44 ^b
M ₃	19.33 ^e	54.00 ^{abc}	44.00 ^d	39.11 ^c
GA ₃ Means	40.22 ^b	49.11 ^a	51.55 ^a	
Significance			LSD _(0.05)	
GA ₃	P<0.0000			2.98
Substrate	P<0.0000			2.98
GA ₃ ×Substrate	P<0.0000			5.17
<i>Trend vs GA₃ treatments</i>				
Linear	P<0.0000		Quadratic	P<0.0178

Table 2. Mean germination time as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	43.66 ^{bcd}	41.46 ^d	42.96 ^d	42.36 ^b
M ₂	45.30 ^{abc}	43.15 ^{cd}	47.32 ^a	45.25 ^a
M ₃	43.53 ^{bcd}	42.53 ^{cd}	46.19 ^{ab}	44.08 ^a
GA ₃ Means	44.16 ^a	42.38 ^b	45.16 ^a	
Significance			LSD _(0.05)	
GA ₃	P<0.0080			1.6395
Substrate	P<0.0063			1.6395
GA ₃ ×Substrate	P<0.1700			2.8397
<i>Trend vs GA₃ treatments</i>				
Linear	P<0.2173		Quadratic	P<0.0036

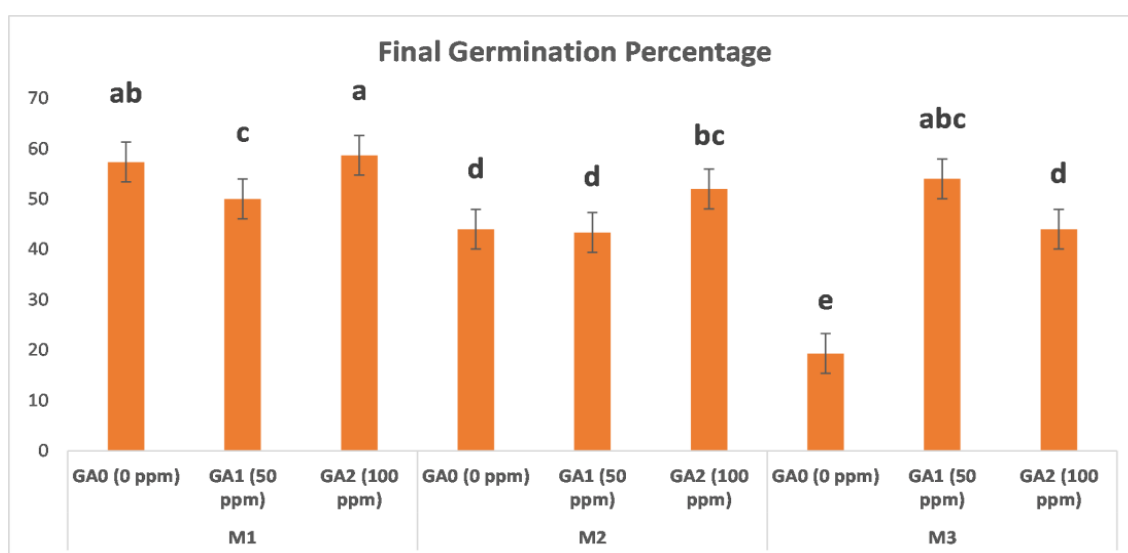


Fig. 2. Effect of gibberellic acid and substrate on final germination percentage

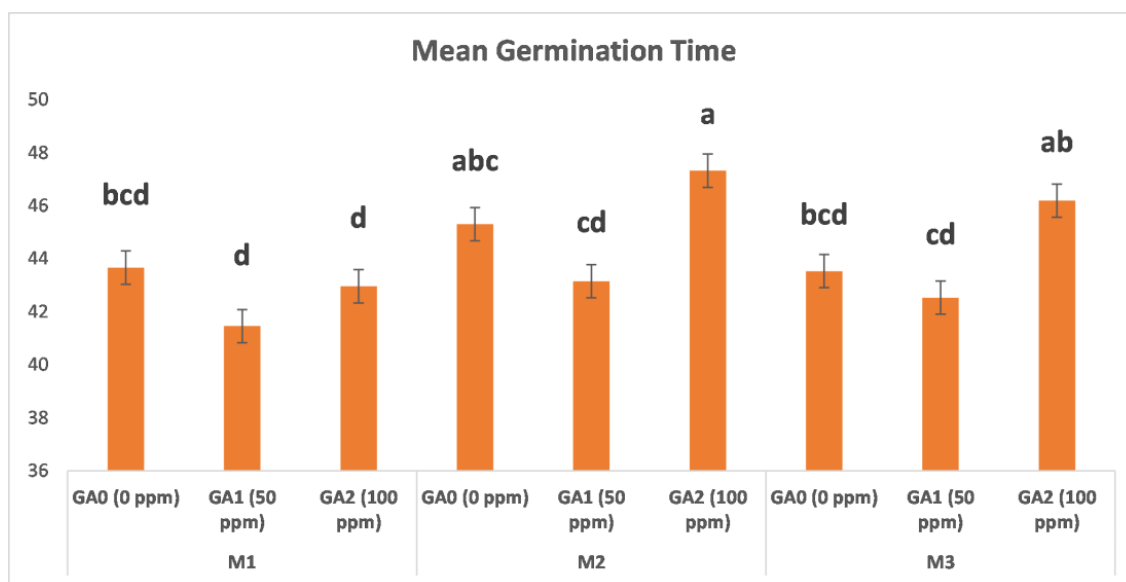


Fig. 3. Effect of gibberellic acid and substrate on mean germination time

any GA₃ pre-treatment (0 ppm) yielded the lowest GI (56.67) (Table 3). In contrast, no significant linear or quadratic relationship was observed between GA₃ concentrations and GI, implying that changes in GA₃ concentrations did not produce a significant trend in GI values.

The analysis indicated that both the substrate, GA₃, and their interactions had a substantial impact on the seed GRI. Among the substrate treatments, M₁ and M₃ yielded the maximum (70.19) and minimum (40.90) seed GRI, respectively, exhibiting significant differences (Table 4). Notably, a GA₃ concentration of 50 ppm (GA₁) resulted in the highest GRI (58.07), whereas GA₀ (0 ppm GA₃) displayed the lowest value (43.64), exhibiting significant disparities in comparison to the control. This represented a notable 24.84% increment in GRI (Table 4). The highest (75.01) and lowest (19.86) GRIs were observed in the interaction involving GA₂ (100 ppm GA₃) when paired with M₁, and GA₀ (0 ppm GA₃) coupled with M₃, respectively. As presented in Table 4, the M₁ treatment demonstrated greater efficacy in combination with GA₂ than GA₁, particularly in terms of enhancing GRI. Moreover, the GRI values displayed a significant quadratic and positive linear response in relation to varying GA₃ concentrations, as illustrated in Fig. 5.

The study revealed that GA₃, substrate, and the interaction between substrate and GA₃ significantly influenced the fresh weight of the

seedlings. The maximum SFW of 7.98 g was achieved with M₁ substrate, while the minimum SFW of 6.37 g was observed with M₃ substrate. In terms of GA₃ treatments, the highest SFW of 8.25 g was obtained with GA₁ (50 ppm), and the lowest SFW of 5.33 g was associated with GA₀ (0 ppm). Significant differences were noted between M₁ and M₂ as well as M₃, and between GA₁ and GA₀ treatments, as indicated in Table 5. GA₁ treatment resulted in a notable 35.39% increase in SFW compared to GA₀. Among the interaction effects, the highest SFW (8.92 g) was observed when seeds were treated with GA₁ and sown in the M₃ medium. Furthermore, the analysis indicated a significant positive linear relationship between fresh seedling weight and GA₃ concentrations, suggesting that higher concentrations of GA₃ were associated with greater seedling weight, as detailed in Table 5.

The data presented in Table 6 indicates that the various substrate treatments did not exert a significant influence on SDW. However, both the GA₃ treatments and their interaction with substrate exhibited a notable impact on SDW. Although GA₂ treatment yielded the highest SDW of 3.95 g, this value did not exhibit a significant difference from that of GA₁. On the other hand, the lowest SDW of 2.46 g was observed in the case of GA₀ treatment, demonstrating an approximate 38% increase compared to GA₀ (Table 6). In terms of interactions, seeds that were initially treated with GA₂ and sown in M₂ substrate demonstrated the maximum SDW

(4.78 g). Conversely, seeds that were not treated with GA₃ (GA₀) and were sown in the M₃ substrate exhibited the lowest SDW of 1.022 g Table 6. The relationship between SDW and GA₃

concentrations displayed a positive correlation, indicating that higher concentrations of GA₃ were associated with increased SDW.

Table 3. Germination index as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	263.33 ^{ab}	215.33 ^{bc}	274.00 ^a	250.88 ^a
M ₂	103.67 ^{ef}	135.00 ^{de}	70.00 ^f	102.88 ^b
M ₃	56.67 ^f	167.00 ^{cd}	125.33 ^{de}	116.33 ^b
GA ₃ Means	141.22 ^a	172.44 ^a	156.44 ^a	
<i>Significance</i>				<i>LSD</i> _(0.05)
GA ₃	<i>P</i> <0.1455			31.705
Substrate	<i>P</i> <0.0000			31.705
GA ₃ ×Substrate	<i>P</i> <0.0025			54.914
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.3239		Quadratic	<i>P</i> <0.0870

Table 4. Germination rate index as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	70.36 ^{ab}	65.19 ^{bc}	75.01 ^a	70.19 ^a
M ₂	40.70 ^d	48.04 ^d	40.31 ^d	43.02 ^b
M ₃	19.86 ^e	60.99 ^c	41.86 ^d	40.90 ^b
GA ₃ Means	43.64 ^c	58.07 ^a	52.39 ^b	
<i>Significance</i>				<i>LSD</i> _(0.05)
GA ₃	<i>P</i> <0.0000			4.9111
Substrate	<i>P</i> <0.0000			4.9111
GA ₃ ×Substrate	<i>P</i> <0.0000			8.5063
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0016		Quadratic	<i>P</i> <0.0001

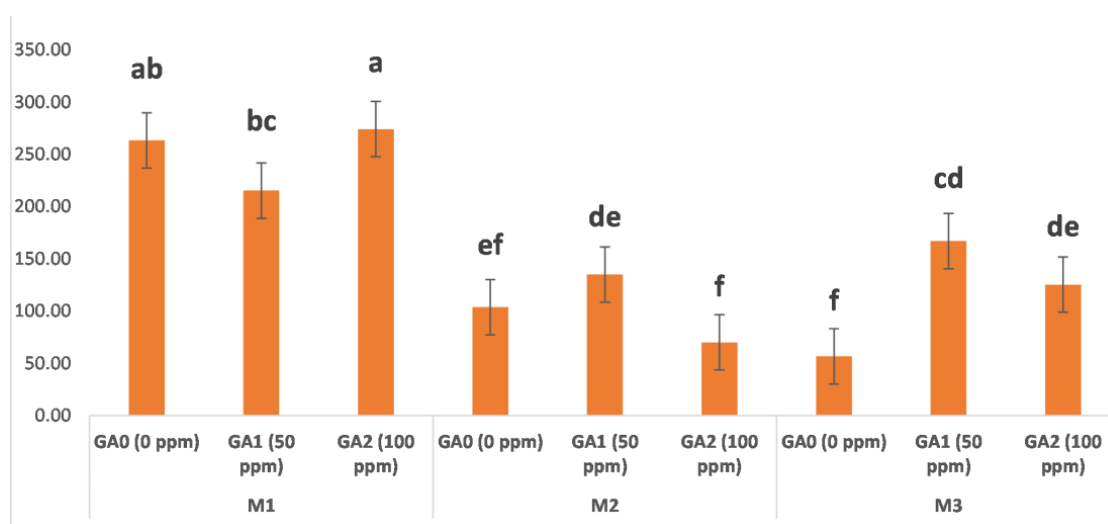


Fig. 4. Effect of gibberellic acid and substrate on germination index

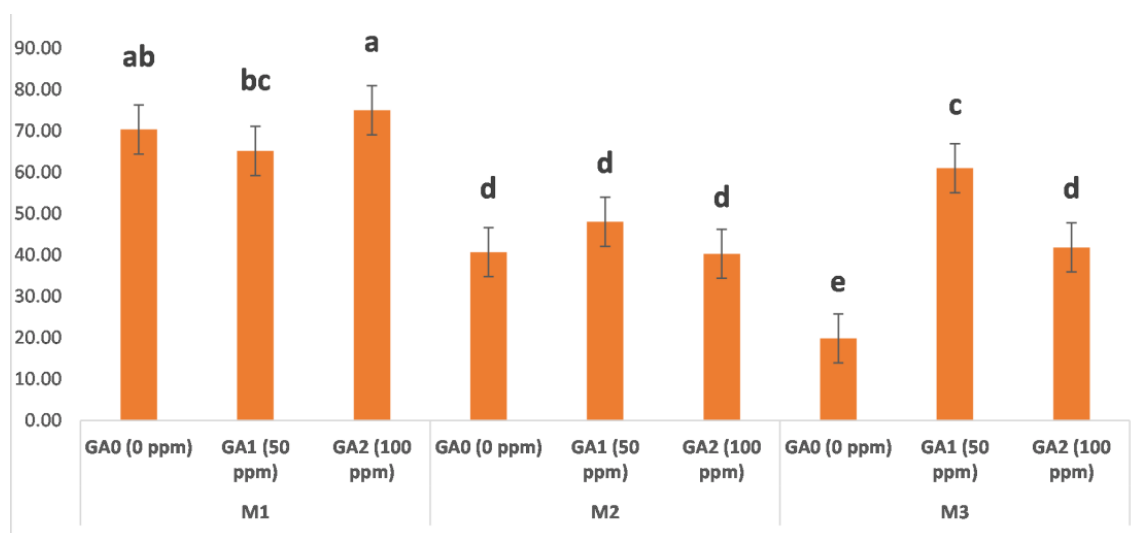


Fig. 5. Effect of gibberellic acid and substrate on germination rate index

Table 5. Seedling fresh weight as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	8.32 ^{ab}	6.88 ^{abc}	8.74 ^a	7.98 ^a
M ₂	5.52 ^c	6.21 ^{bc}	7.97 ^{ab}	6.57 ^b
M ₃	2.14 ^d	8.92 ^a	8.05 ^{ab}	6.37 ^b
GA ₃ Means	5.33 ^b	7.34 ^a	8.25 ^a	
<i>Significance</i>				<i>LSD</i> _(0.05)
GA ₃	<i>P</i> <0.0008			1.3310
Substrate	<i>P</i> <0.0409			1.3310
GA ₃ ×Substrate	<i>P</i> <0.0010			2.3054
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0003			Quadratic <i>P</i> <0.3290

Table 6. Seedling dry weight as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	3.88 ^{ab}	3.13 ^b	3.90 ^{ab}	3.63 ^a
M ₂	2.50 ^{bc}	2.86 ^b	4.78 ^a	3.38 ^a
M ₃	1.02 ^c	3.99 ^{ab}	3.16 ^b	2.72 ^a
GA ₃ Means	2.46 ^b	3.33 ^{ab}	3.95 ^a	
<i>Significance</i>				<i>LSD</i> _(0.05)
GA ₃	<i>P</i> <0.0130			0.9290
Substrate	<i>P</i> <0.1328			0.9290
GA ₃ ×Substrate	<i>P</i> <0.0170			1.6090
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0038			Quadratic <i>P</i> <0.7552

As illustrated in Table 7, the various substrate and GA₃ treatments, along with their interaction, exhibited significant influences on seedling length (SL). In terms of substrate effects, the highest SL of 203.85 cm was recorded with the M₁ treatment, while the lowest length of 135.72

cm was observed with the M₃ treatment. Within the GA₃ treatments, the maximum SL of 189.46 cm resulted from GA₂, while the minimum length of 140.04 cm was associated with GA₀. Notably, GA₂ treatment led to a 26% increase in SL compared to GA₀ Table 7. Examining interaction

effects, the highest SL of 219.80 cm was achieved when seeds were treated with GA₂ and sown in M₁ substrate. In contrast, seeds that were not treated with GA₃ and were sown in M₃ substrate exhibited the lowest SL of 64.80 cm Table 7. Furthermore, the analysis demonstrated a significant positive linear correlation between SL and GA₃ concentrations, implying that higher concentrations of GA₃ were linked to increased SL, as outlined in Table 7.

The analysis revealed that both the main effects of soil substrate and GA₃, as well as their interactions, had a substantial impact on SVI. The highest SVI value of 113647.889 was recorded with M₁ substrate, while the lowest value of 61009.378 was observed with M₃ substrate, demonstrating significant differences Table 8. Regarding GA₃ treatments, the highest SVI of 99703.4 was achieved with GA₂, while the lowest SVI of 65636.387 was associated with GA₀ (control). This indicated an approximate 34% increase in SVI when comparing GA₂ to the control GA₀ treatment Table 8. The interaction

effects demonstrated that the combination of GA₂ with M₁ and GA₀ with M₃ led to the highest SVIs of 129192.60 and the lowest SVI of 13008.53, respectively Table 8.

4. DISCUSSION

The outcomes of this study underscore the substantial influence exerted by the selection of growth substrates, the application of GA₃, and their intricate interplay on the germination percentage of Chilgoza pine seeds. While both factors play a role, the impact of the substrate appears to be more pronounced. Notably, the medium comprised of soil, sand, and FYM in equal proportions yielded the highest germination rates. In contrast, the application of GA₃ exhibited a gradual increase in germination percentage with escalating concentrations. Specifically, the utilization of 100 ppm GA₃ resulted in an approximately 22% augmentation in FGP when compared to 0 ppm GA₃. The chosen growth medium contributes to conditions of aeration and nutrition, creating a favorable

Table 7. Seedling length as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	203.83 ^{ab}	187.91 ^b	219.80 ^a	203.85 ^a
M ₂	151.48 ^d	146.41 ^d	187.80 ^b	161.90 ^b
M ₃	64.80 ^e	181.58 ^{bc}	160.79 ^{cd}	135.72 ^c
GA ₃ Means	140.04 ^c	171.97 ^b	189.46 ^a	
	<i>Significance</i>		<i>LSD</i> _(0.05)	
GA ₃	<i>P</i> <0.0000		15.014	
Substrate	<i>P</i> <0.0000		15.014	
GA ₃ ×Substrate	<i>P</i> <0.0000		26.006	
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0000	Quadratic	<i>P</i> <0.2566	

Table 8. Seedling vigor index as affected by gibberellic acid, substrate and their interaction

Substrate	Gibberellic acid			Means for Substrate
	GA ₀ (0 ppm)	GA ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	117136.66 ^{ab}	946143.98 ^c	1291926.02 ^a	113647.88 ^a
M ₂	66763.96 ^d	63501.33 ^d	985098.67 ^{bc}	76258.38 ^b
M ₃	130085.33 ^e	986118.67 ^{bc}	71407.73 ^d	61009.37 ^c
GA ₃ Means	65636.38 ^c	85575.86 ^b	99703.40 ^a	
	<i>Significance</i>		<i>LSD</i> _(0.05)	
GA ₃	<i>P</i> <0.0001		12038	
Substrate	<i>P</i> <0.0000		12038	
GA ₃ ×Substrate	<i>P</i> <0.0000		20851	
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0000	Quadratic	<i>P</i> <0.5628	

environment for seed germination. Concurrently, GA₃ functions by enhancing the activities of hydrolyzing enzymes responsible for starch degradation, a pivotal process that contributes to improved seed germination [28]. The intricate interplay between these factors highlights their combined role in fostering successful germination outcomes for Chilgoza pine seeds. The growth medium's role in seed germination cannot be understated, as it creates a conducive environment that facilitates crucial processes. The carefully balanced combination of medium ensures adequate aeration and nutrient availability for the Chilgoza pine seeds. Proper aeration is essential for seed respiration, allowing the embryo to utilize stored energy efficiently during germination [29]. Moreover, the medium's nutrient content provides the essential elements and compounds required for seedling growth, enabling robust root and shoot development [30]. In parallel, the application of GA₃, a synthetic plant growth regulator, proved to be a pivotal factor in augmenting germination. GA₃ is involved in various physiological processes, and in the context of seed germination, it significantly affects the activities of hydrolyzing enzymes. These enzymes play a crucial role in breaking down stored starch reserves in the seed's endosperm into simpler sugars, which serve as the primary source of energy for the developing seedling [31]. By promoting starch degradation, GA₃ ensures a quick and efficient mobilization of energy, accelerating the germination process and enabling the seedling to establish itself with vigor [32]. Our findings are in line with those reported by Galston & Davis [33], Verma & Tandon [34], and Bahardwaj et al. [21].

The study revealed that two specific treatments, 50 ppm GA₃ and M₁, were equally effective in reducing the mean germination time of Chilgoza pine seeds. This implies that both the optimal concentration of GA₃ (50 ppm) and the well-balanced substrate (M₁) facilitated quicker seed germination, hastening the process compared to the control treatment without GA₃ (0 ppm GA₀). However, an interesting finding emerged when examining the effect of a higher GA₃ concentration. The application of 100 ppm GA₃ treatment led to a noticeable delay in the germination time when compared to the control treatment. This delay can be attributed to the higher concentration of GA₃, which might have exerted an inhibitory effect on the germination process. This phenomenon aligns with findings from Hu et al. [35], suggesting that elevated GA₃ concentrations could potentially impede the

timely onset of germination. Plant growth regulators like GA₃ can have varying effects on seed germination and growth, depending on their concentration. At an optimal concentration, GA₃ can promote and accelerate germination by stimulating the activity of hydrolyzing enzymes, as discussed earlier. However, when applied at higher concentrations, GA₃ may have an adverse impact on seed germination. Excess GA₃ can disrupt hormonal balances and interfere with physiological processes, leading to delays in germination and reduced seedling vigor [36]. The observed delay in germination with 100 ppm GA₃ underscores the importance of careful dosing when using growth regulators like GA₃. It highlights the need for precise application rates to achieve the desired effects without causing unintended negative consequences. Finding the right balance in GA₃ concentration is crucial to optimizing seed germination and ensuring the successful establishment of healthy seedlings.

The results of our study revealed an intriguing finding concerning the influence of GA₃ and the substrate on the seed GI. Interestingly, when GA₃ was applied alone, it did not show a significant effect on the seed GI. This suggests that GA₃ alone might not be sufficient to promote or accelerate the germination process in Chilgoza pine seeds. In contrast, the choice of substrate played a crucial role in influencing the seed GI. Different combinations of substrates had a considerable impact on the GI, highlighting the significance of the substrate in facilitating successful seed germination. These findings underscore the complex nature of seed germination and emphasize that a combination of factors, such as the substrate and plant growth regulators like GA₃, may be necessary to optimize the germination process effectively. Indeed, the findings suggest that GA₃ plays a crucial role during the initial phases of germination, particularly in breaking dormancy and initiating the process. However, as germination progresses, the focus shifts towards the importance of the growth substrate. Subsequently, the substrate becomes instrumental in facilitating later stages of germination, such as radicle protrusion and emergence. This underscores the dynamic interplay between GA₃ and the growth substrate, each contributing distinctively to different phases of the germination process. GA₃ plays a critical role during the early stages of germination. It seems to be instrumental in breaking dormancy and initiating the germination process [37].

However, as the germination progresses, the choice of the substrate become increasingly important, particularly during later stages like radicle protrusion and seedling emergence. The application of GA₃ appears to be essential for overcoming dormancy barriers and triggering the initial stages of germination. It likely stimulates the activity of hydrolyzing enzymes responsible for breaking down stored reserves within the seed, facilitating the embryo's growth and metabolic activation [38]. As germination advances, the focus shifts to the role of the substrate. The substrate's composition, a combination of soil, sand, and FYM in our study, seems crucial for providing the necessary physical support, aeration, and essential nutrients required for radicle protrusion and seedling development. The substrate becomes the foundation for the successful emergence and establishment of the seedlings, ensuring they have access to vital resources for growth and survival.

The application of GA₃ treatment notably led to a significant increase in the SFW, surpassing the effects of substrate treatments and their interactions. This augmentation in fresh weight can be attributed to the stimulatory impact of GA₃ on critical physiological processes within the seedlings, specifically cell enlargement and the generation of turgor pressure. The work of Sprangers et al. [39] supports this notion, highlighting the role of GA₃ in fostering these essential growth mechanisms within plants. GA₃ is known to act as a growth-promoting hormone, influencing various physiological processes in plants. It stimulates cell elongation and division, leading to increased cell size and overall tissue growth [40]. Additionally, GA₃ enhances turgor pressure, the internal pressure exerted by cell contents against the cell wall, which plays a crucial role in maintaining cell shape and rigidity. By inducing cell enlargement and boosting turgor pressure, GA₃ facilitates greater water uptake, nutrient absorption, and overall metabolic activity within the seedlings Shigeyama et al. [41] This, in turn, translates into the observed increase in fresh weight, as the treated seedlings exhibit robust growth and vigor compared to those without GA₃ treatment. Our research outcomes are consistent with the findings reported by Sudhakar et al. [42] and Lavania et al. [43]. We observed a positive linear relationship between GA₃ concentrations and various parameters such as FGP, GRI, SFW, SDW, SL, and SVI. This suggests that as the concentration of GA₃ increases, these parameters also tend to

increase, showing a direct correlation. Additionally, positive quadratic trends were only evident in the case of FGP and MGT, indicating that the response of these two parameters to increasing GA₃ concentrations follows a curvilinear pattern. While our study provides a general overview of the relationship between different parameters and GA₃ concentrations, future investigations should consider incorporating a wider range of GA₃ concentrations with narrower intervals. This approach would enhance the precision and accuracy of the response analysis, providing a more detailed understanding of the impact of GA₃ on various aspects of seed germination and seedling growth [44].

5. CONCLUSIONS

The findings of this study underscore the considerable impact of both the growth substrate and GA₃, as well as their intricate interaction, on seed germination and seedling growth. Notably, the most favorable treatment emerged from the interaction involving 100 ppm GA₃ and the M₁ substrate. While the main effects of the substrate appeared to be more influential than GA₃ on seed germination, the simple effects of GA₃ exerted a more pronounced influence on the vegetative growth of the seedlings. Interestingly, all the parameters studied exhibited a linear response to varying levels of GA₃ concentration. However, the trends were quadratic in the case of FGP and MGT, indicating a more complex relationship in these specific instances. For future investigations, it is recommended to explore the impact of various levels of GA₃ on the vegetative growth of seedlings, considering different application methods such as ground or foliar application. This could provide further insights into the potential benefits of GA₃ in enhancing the growth and development of seedlings.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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