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

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 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 (GA_3) 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): M_1 (1:1:1), M_2 (1:1/2:1/2), M_3 (1:1/3:2/3). Additionally, three concentrations of GA_3 (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 GA_3 solution for 6 hours and then planted in a medium with equal proportions of soil, sand, and FYM. The effects of GA_3 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 GA_3 concentrations exhibited a notable linear trend, whereas the relationship between FGP and MGT with GA_3 concentrations showed a significant quadratic pattern. This suggests that GA_3 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 GA_3 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 GA_3 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

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 (Shalizi *et al.,* 2018). 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 (Kumar *et al.,* 2013). 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 (Bhattacharyya *et al.,* 1988). 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 (Rahman *et al.,* 2021). *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 (Negi & Subramani, 2015).

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 (Singh *et al.*, 2021). 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 (Awan & Pettenella, 2017). 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] (UNEP, 2008). 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 (Malik & Shamet, 2008). This important species has vanished or been exterminated due to severe biotic interference and a lack of regeneration (Kumar *et al.*, 2014). 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 (Vahdati *et al.*, 2012). Researchers, conservationists, and local communities are joining forces to implement measures that promote Chilgoza pine regeneration (Asher & Bhandari, 2021). Protective measures are being introduced to reduce the gathering of cones from mature trees, allowing them to contribute to seed dispersal naturally (Qiu *et al.*, 2022). 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 (Lotfi *et al.*, 2019).

Growth medium significantly impacts seed germination, growth, and nursery seedling quality (Wawo *et al.*, 2020). 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 (Hubbard *et al.*, 2012). 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 (Cardoso, 2012). 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 (Almeida *et al.,* 2014; Shen *et al.,* 2015). 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.* (2021) demonstrated the significant influence of both soil media and GA_3 on the germination and seedling growth of distinct forest tree seeds. Additionally, Bhardwaj *et al.* (1986) 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 roxburghi*. Several investigations have unveiled noteworthy impacts of GA*3* on seed germination. For instance, Amri's study (2010) revealed the concentration-dependent effect of GA_3 on the seed germination of *Terminalia sericea*. Similarly, Kumar *et al.* (2014) identified a positive correlation between GA_3 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 (Luna, 2008). 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 GA_3 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 GA_3 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.

Materials and Methods

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 km^{-2}. 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℃; the wintertime environment is chilly, with low temperatures ranging from -10 to -20℃. 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 ($CaCO_3$). Soil pH is often high, ranging from 8.00 to 8.50. Tillage and nutrients are well-tolerated by these soils.

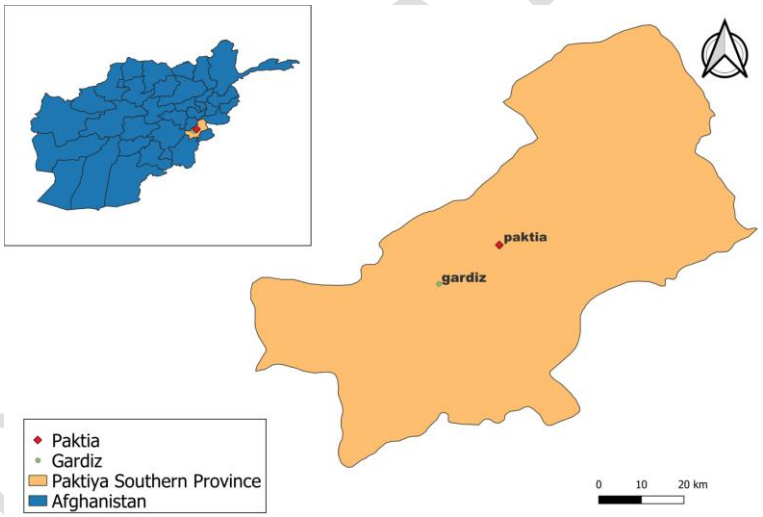

Fig.1. Map of the study area.

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 GA_3 were sourced from the local market, available in powdered form. To create various GA_3 concentrations (control - GA_0 [0 ppm], GA_1 [50 ppm], and GA_2 [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: M_1 (1:1:1), M_2 (1:1/2:1/2), and M_3 (1:1/3:2/3). The procedure began with treating the seeds for a six-hour duration with diverse GA_3 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 28^{th} and 68^{th} 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℃ 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:

Final Germination Percentage (FGP)

The final germination percentage was computed according to Kumar (2012) using the formula;

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

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

Mean Germination Time (MGT)

The mean germination time was computed according to Maguire (1962) using the formula;

$$MGT = \frac{\sum F*X}{\sum F} \qquad (2)$$

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

Germination Index (GI)

The germination index was calculated according to (Bewley et al., 2013) using the formula;

$$GI = (10 \text{ x } N1) + (9 \text{ x } N2) + \cdots . (1 \text{ x } N10) \qquad (3)$$

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

Germination Rate Index (GRI)

The germination rate index was calculated according to Maguire (1962) using the formula;

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

Where P_1= percentage of seeds germinated on the 1^{st} day, P_2= percentage of seeds germinated on the 2^{nd} day, P3 is the percentage of germination on the 3^{rd} day, and x= number of days after seed sowing.

Seedling Vigor Index (SVI)

The seedling vigor index was computed according to Abdul- Baki and Anderson (1973) using the formula;

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

Where SDW= seedling dry weight and FGP= final germination parentage.

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.

Results

The combination of substrate and GA_3 significantly impacted the FGP, as indicated by the results in Table 1. Specifically, treatments M_1 and GA_2 yielded FGP rates of 55.33% and 51.55% respectively, while the combined treatment of M_1 and GA_2 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, GA_3, and their interaction, as illustrated in Figure 2. Upon closer examination through simple effects analysis, it was evident that the highest GA_3 concentration (100 ppm) was not as effective as the soil substrate (M_1) in promoting germination. However, the interaction between GA_3 and substrate showed potential for enhancing germination (Table 1). The data in Table 1 further illustrates that as GA_3 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 GA_3 concentrations demonstrated a notably positive linear and quadratic pattern.

The analysis of data revealed that both substrate and GA_3 treatments had a notable impact on MGT, as detailed in Table 2. Conversely, the interaction between substrate and GA_3 did not demonstrate a significant influence on MGT. Among the substrate treatments, M_1 displayed the shortest MGT at 42.36 days, while M_2 exhibited the longest MGT at 45.25 days, indicating a reduction of 6.5% in MGT. The disparity between M_1 and M_2 was statistically significant, as depicted in Figure 3. In terms of GA_3 treatments, the briefest MGT (42.38 days) was observed with GA_1 (50 ppm), while the longest MGT (45.16 days) was associated with GA_2 (100 ppm), resulting in a comparable 6.5% decrease (Table 2). Notably, significant differences were observed between GA_1 and GA_2 treatments, while no statistically significant difference was observed between GA_0 (0 ppm) and GA_2 (100 ppm) (Table 2). Furthermore, the relationship between MGT and GA_3 concentrations exhibited a significant quadratic pattern, implying a non-linear response to varying GA_3 concentrations.

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
Trend vs GA_3 treatments				
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
Trend vs GA_3 treatments			
Linear	*P*<0.2173	Quadratic	*P*<0.0036

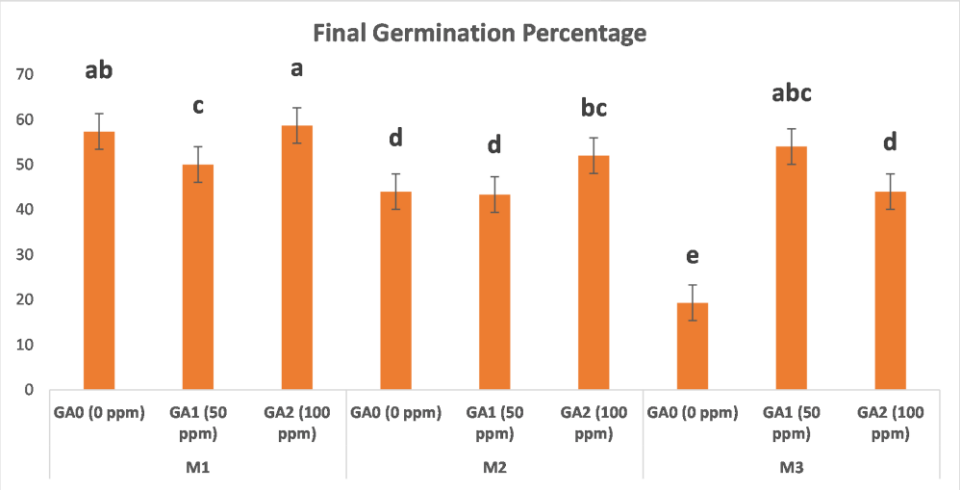

Fig.2. Effect of gibberellic acid and substrate on final germination percentage.

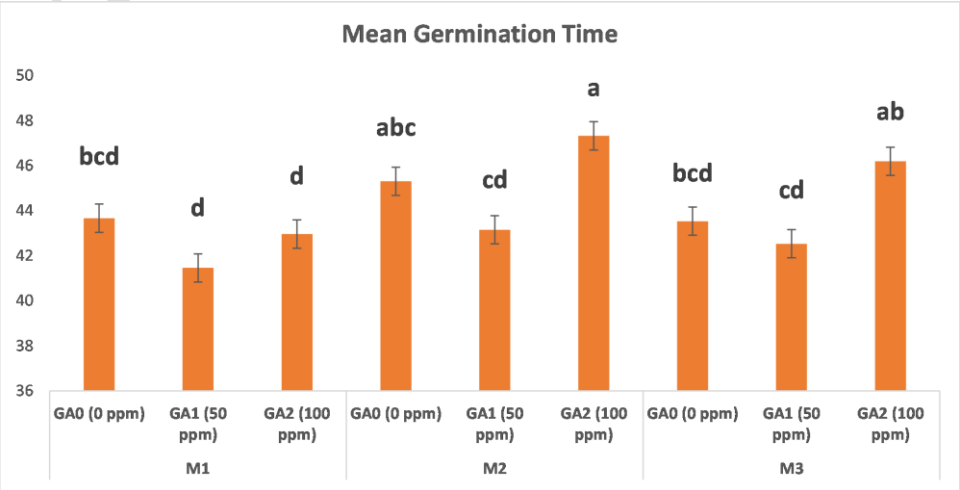

Fig.3. Effect of gibberellic acid and substrate on mean germination time.

The analysis of variance revealed a significant interaction effect between the substrate and GA_3 on GI, as depicted in Figure 4. The greatest GI value (250.889) was achieved from seeds cultivated in M_1 soil substrate, while the lowest GI (102.88) was observed in seeds grown in M_2 soil substrate. This difference between M_1 and M_2 was statistically significant, as indicated in Table 3. Notably, when seeds were pre-treated with 100 ppm GA_3 (GA_2) and subsequently sown in M_1 soil substrate, the highest GI (274) was attained. Conversely, seeds sown in M_3 without any GA_3 pre-treatment (0 ppm) yielded the lowest GI (56.67) (Table 3). In contrast, no significant linear or quadratic relationship was observed between GA_3 concentrations and GI, implying that changes in GA_3 concentrations did not produce a significant trend in GI values.

The analysis indicated that both the substrate, GA_3, and their interactions had a substantial impact on the seed GRI. Among the substrate treatments, M_1 and M_3 yielded the maximum (70.19) and minimum (40.90) seed GRI, respectively, exhibiting significant differences (Table 4). Notably, a GA_3 concentration of 50 ppm (GA_1) resulted in the highest GRI (58.07), whereas GA_0 (0 ppm GA_3) 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 GA_2 (100 ppm GA_3) when paired with M_1, and GA_0 (0 ppm GA_3) coupled with M_3, respectively. As presented in Table 4, the M_1 treatment demonstrated greater efficacy in combination with GA_2 than GA_1, particularly in terms of enhancing GRI. Moreover, the GRI values displayed a significant quadratic and positive linear response in relation to varying GA_3 concentrations, as illustrated in Figure 5.

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}	

Significance		$LSD_{(0.05)}$
GA_3	$P<0.1455$	31.705
Substrate	$P<0.0000$	31.705
GA_3×Substrate	$P<0.0025$	54.914

Trend vs GA_3 treatments			
Linear	$P<0.3239$	Quadratic	$P<0.0870$

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}	

Significance		$LSD_{(0.05)}$
GA_3	$P<0.0000$	4.9111
Substrate	$P<0.0000$	4.9111

GA_3×Substrate	*P*<0.0000			8.5063
Trend vs GA_3 treatments				
Linear	*P*<0.0016	Quadratic	*P*<0.0001	

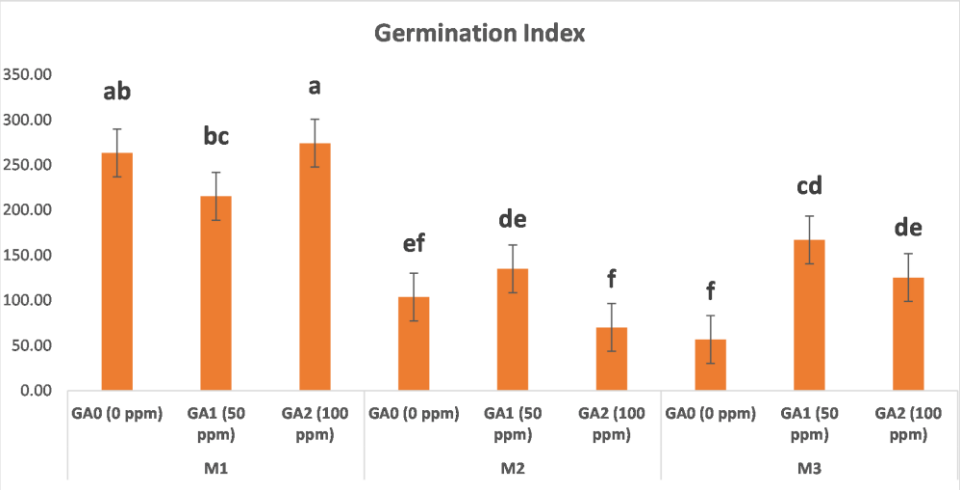

Fig.4. Effect of gibberellic acid and substrate on germination index.

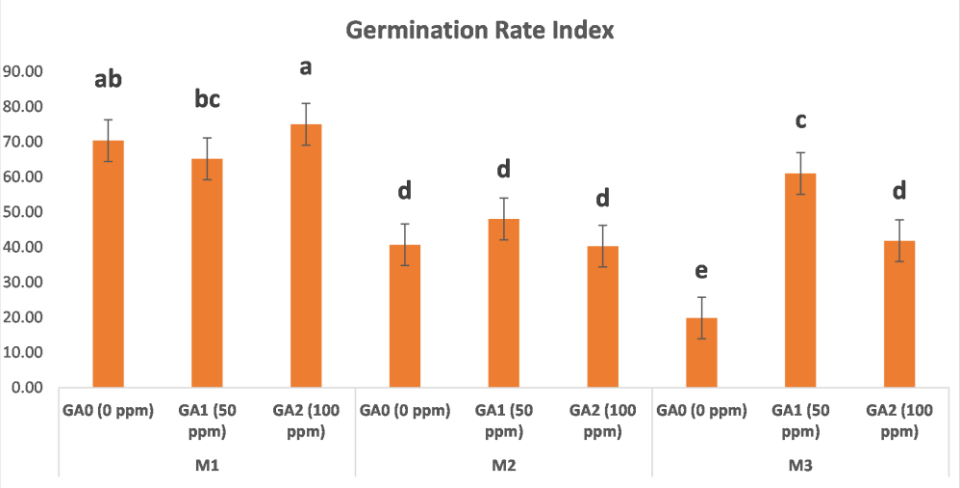

Fig.5. Effect of gibberellic acid and substrate on germination rate index.

The study revealed that GA_3, substrate, and the interaction between substrate and GA_3 significantly influenced the fresh weight of the seedlings. The maximum SFW of 7.98 g was achieved with M_1 substrate, while the minimum SFW of 6.37 g was observed with M_3 substrate. In terms of GA_3 treatments, the highest SFW of 8.25 g was obtained with GA_1 (50 ppm), and the lowest SFW of 5.33 g was associated with GA_0 (0 ppm). Significant differences were noted between M_1 and M_2 as well as M_3, and between GA_1 and GA_0 treatments, as indicated in Table 5. GA_1 treatment resulted in a notable 35.39% increase in SFW compared to GA_0. Among the interaction effects, the highest SFW (8.92 g) was observed when seeds were treated with GA_1 and sown in the M_3 medium. Furthermore, the analysis indicated a significant positive linear relationship between fresh seedling weight and GA_3 concentrations, suggesting that higher concentrations of GA_3 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 GA_3 treatments and their interaction with substrate exhibited a notable impact on SDW. Although GA_2 treatment yielded the highest SDW of 3.95 g, this value did not exhibit a significant difference from

that of GA_1. On the other hand, the lowest SDW of 2.46 g was observed in the case of GA_0 treatment, demonstrating an approximate 38% increase compared to GA_0 (Table 6). In terms of interactions, seeds that were initially treated with GA_2 and sown in M_2 substrate demonstrated the maximum SDW (4.78 g). Conversely, seeds that were not treated with GA3 (GA_0) and were sown in the M_3 substrate exhibited the lowest SDW of 1.022 g (Table 6). The relationship between SDW and GA_3 concentrations displayed a positive correlation, indicating that higher concentrations of GA_3 were associated with increased SDW.

As illustrated in Table 7, the various substrate and GA_3 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 M_1 treatment, while the lowest length of 135.72 cm was observed with the M_3 treatment. Within the GA_3 treatments, the maximum SL of 189.46 cm resulted from GA_2, while the minimum length of 140.04 cm was associated with GA_0. Notably, GA_2 treatment led to a 26% increase in SL compared to GA_0 (Table 7). Examining interaction effects, the highest SL of 219.80 cm was achieved when seeds were treated with GA_2 and sown in M_1 substrate. In contrast, seeds that were not treated with GA_3 and were sown in M_3 substrate exhibited the lowest SL of 64.80 cm (Table 7). Furthermore, the analysis demonstrated a significant positive linear correlation between SL and GA_3 concentrations, implying that higher concentrations of GA_3 were linked to increased SL, as outlined in Table 7.

The analysis revealed that both the main effects of soil substrate and GA_3, as well as their interactions, had a substantial impact on SVI. The highest SVI value of 113647.889 was recorded with M_1 substrate, while the lowest value of 61009.378 was observed with M_3 substrate, demonstrating significant differences (Table 8). Regarding GA_3 treatments, the highest SVI of 99703.4 was achieved with GA_2, while the lowest SVI of 65636.387 was associated with GA_0 (control). This indicated an approximate 34% increase in SVI when comparing GA_2 to the control GA_0 treatment (Table 8). The interaction effects demonstrated that the combination of GA_2 with M_1 and GA_0 with M_3 led to the highest SVIs of 129192.60 and the lowest SVI of 13008.53, respectively (Table 8).

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}	

Significance		$LSD_{(0.05)}$
GA_3	$P<0.0008$	1.3310
Substrate	$P<0.0409$	1.3310
GA_3×Substrate	$P<0.0010$	2.3054

Trend vs GA_3 treatments			
Linear	$P<0.0003$	Quadratic	$P<0.3290$

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}	

Significance				$LSD_{(0.05)}$
GA_3	*P*<0.0130			0.9290
Substrate	*P*<0.1328			0.9290
GA_3×Substrate	*P*<0.0170			1.6090
Trend vs GA_3 treatments				
Linear	*P*<0.0038	Quadratic	*P*<0.7552	

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}	
	Significance		$LSD_{(0.05)}$	
GA_3	*P*<0.0000		15.014	
Substrate	*P*<0.0000		15.014	
GA_3×Substrate	*P*<0.0000		26.006	
Trend vs GA_3 treatments				
Linear	*P*<0.0000	Quadratic	*P*<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}	
Significance			$LSD_{(0.05)}$	
GA_3	*P*<0.0001		12038	
Substrate	*P*<0.0000		12038	
GA_3×Substrate	*P*<0.0000		20851	
Trend vs GA_3 treatments				
Linear	*P*<0.0000	Quadratic	*P*<0.5628	

Discussion

The outcomes of this study underscore the substantial influence exerted by the selection of growth substrates, the application of GA_3, 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 GA_3 exhibited a gradual increase in germination percentage with escalating concentrations. Specifically, the utilization of 100 ppm GA_3 resulted in an approximately 22% augmentation in FGP when compared to 0 ppm GA_3. The chosen growth medium contributes to conditions of aeration and nutrition, creating a favorable environment for seed germination. Concurrently, GA_3 functions by enhancing the activities of hydrolyzing enzymes responsible for starch

degradation, a pivotal process that contributes to improved seed germination (Nile et al., 2022). 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 (Finch-Savage & Bassel, 2016). Moreover, the medium's nutrient content provides the essential elements and compounds required for seedling growth, enabling robust root and shoot development (Bhatla *et al.,* 2018). In parallel, the application of GA_3, a synthetic plant growth regulator, proved to be a pivotal factor in augmenting germination. GA_3 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 (He *et al.,* 2015). By promoting starch degradation, GA_3 ensures a quick and efficient mobilization of energy, accelerating the germination process and enabling the seedling to establish itself with vigor (Mahakham *et al.,* 207). Our findings are in line with those reported by Galston & Davis (1969), Verma & Tandon (1988), and Bahardwaj *et al.* (1986).

The study revealed that two specific treatments, 50 ppm GA_3 and M_1, were equally effective in reducing the mean germination time of Chilgoza pine seeds. This implies that both the optimal concentration of GA_3 (50 ppm) and the well-balanced substrate (M_1) facilitated quicker seed germination, hastening the process compared to the control treatment without GA_3 (0 ppm GA_0). However, an interesting finding emerged when examining the effect of a higher GA_3 concentration. The application of 100 ppm GA_3 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 GA_3, which might have exerted an inhibitory effect on the germination process. This phenomenon aligns with findings from Hu *et al.* (2017), suggesting that elevated GA_3 concentrations could potentially impede the timely onset of germination. Plant growth regulators like GA_3 can have varying effects on seed germination and growth, depending on their concentration. At an optimal concentration, GA_3 can promote and accelerate germination by stimulating the activity of hydrolyzing enzymes, as discussed earlier. However, when applied at higher concentrations, GA_3 may have an adverse impact on seed germination. Excess GA_3 can disrupt hormonal balances and interfere with physiological processes, leading to delays in germination and reduced seedling vigor (Reed *et al.,* 2022). The observed delay in germination with 100 ppm GA_3 underscores the importance of careful dosing when using growth regulators like GA_3. It highlights the need for precise application rates to achieve the desired effects without causing unintended negative consequences. Finding the right balance in GA_3 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 GA_3 and the substrate on the seed GI. Interestingly, when GA_3 was applied alone, it did not show a significant effect on the seed GI. This suggests that GA_3 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 GA_3, may be necessary to optimize the germination process effectively. Indeed, the findings suggest that GA_3 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 GA_3 and the growth substrate, each contributing distinctively to different phases of the germination process. GA_3 plays a critical role during the early stages of germination. It seems to be instrumental in breaking dormancy and initiating the germination process (Gong *et al.*, 2021). 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 GA_3 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 (Garcia *et al.*, 2021). 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 GA_3 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 GA_3 on critical physiological processes within the seedlings, specifically cell enlargement and the generation of turgor pressure. The work of Sprangers et al. (2020) supports this notion, highlighting the role of GA_3 in fostering these essential growth mechanisms within plants. GA_3 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 (Iqbal *et al.*, 2011). Additionally, GA_3 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, GA_3 facilitates greater water uptake, nutrient absorption, and overall metabolic activity within the seedlings (Shigeyama *et al.*, 2016). This, in turn, translates into the observed increase in fresh weight, as the treated seedlings exhibit robust growth and vigor compared to those without GA_3 treatment. Our research outcomes are consistent with the findings reported by Sudhakar *et al.* (1995) and Lavania *et al.* (2006). We observed a positive linear relationship between GA_3 concentrations and various parameters such as FGP, GRI, SFW, SDW, SL, and SVI. This suggests that as the concentration of GA_3 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 GA_3 concentrations follows a curvilinear pattern. While our study provides a general overview of the relationship between different parameters and GA_3 concentrations, future investigations should consider incorporating a wider range of GA_3 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 GA_3 on various aspects of seed germination and seedling growth.

Conclusions

The findings of this study underscore the considerable impact of both the growth substrate and GA_3, as well as their intricate interaction, on seed germination and seedling growth. Notably, the most favorable treatment emerged from the interaction involving 100 ppm GA_3 and the M_1 substrate. While the main effects of the substrate appeared to be more influential than GA_3 on seed germination, the simple effects of GA_3 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 GA_3 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 GA_3 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 GA_3 in enhancing the growth and development of seedlings.

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