

Sowing media and gibberellic acid influences germination and seedling growth of chilgoza pine (*Pinus gerardiana*. Wall)

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Abstract: *Pinus gerardiana* Wall. is a notable nut-producing pine having essential ecological and economically viable forestry species having restricted distribution in Afghanistan. Natural regeneration of the species is either incredibly poor or nonexistent. Due to irregular and uncommon seed years, problems with dormancy, and other factors, this species' natural habitat renewal is slowed down. Therefore, we investigated the effects of sowing media and gibberellic acid on germination and seedling growth of chilgoza pine. The sowing media were categorized into three types with various proportions of soil, sand, and FYM, viz., M₁ (1:1:1), M₂ (1:1/2:1/2), M₃ (1:1/3:2/3), and three GA₃ concentrations were used, viz., 0, 50, and 100 mg. L⁻¹, for assessing their influence on germination and seedling growth. It was observed that apart from MGT (42.36 days), the higher FGP (58.66%), GI (274), GRI (75.01), SFW (8.74gr), SDW (4.78gr), SL (219.80 mm), SVI (129192602) were obtained when seeds were initially immersed for 6 h at 100 mg. L⁻¹ GA₃ solution and then sown in the medium of equal proportions of soil, sand, and FYM. In terms of simple effects, GA₃ concentrations significantly impacted the vegetative growth of seedlings. In contrast, media effects on seed germination were highly significant, meaning that chilgoza seed contains sufficient gibberellin for germination and is reduced during seedling growth stages. The vegetative growth response of the seedling to the concentrations of GA₃ had a significant linear response, but the response of FGP and MGT against GA₃ concentrations was significantly quadratic, i.e., the use of GA₃ concentrations can stimulate the growth of seedlings. The sowing media had a pronounced effect on FGP and MGT due to nutrients and ventilation. The highest concentration of GA₃ increased the seedling's vegetative growth, so further use may be necessary over time. It is suggested that GA₃ and media may be used to improve chilgoza seed germination and subsequent seedling growth.

Keywords: Ecological, Gibberellic acid, *Pinus gerardiana* Wall, Regeneration, Sowing media

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1. Introduction

Chilgoza pine (*Pinus gerardiana* Wall.) is a significant ecological and commercial tree species found in Afghanistan, Pakistan, and the temperate Himalayan region of India (Shalizi et al., 2018). Chilgoza pine is native forest species that have high economic importance and is valuable to Afghanistan. *Pinus gerardiana* Wall., often known as "chilgoza" or "neoza pine," is a member of the Pinaceae family and was first found in India in 1932 by Captain Gerard, a British officer (Kumar et al., 2013). It is an evergreen tree of small to medium size that ranges from 17 to 27 meters in height and 2 to 4 meters in girth (Bhattacharyya et al., 1988). *Pinus gerardiana* is a species of immense importance in the *Pinus* genus. The species produces edible nuts with high nutritional and economic value, known as *Chilgoza* nuts, in domestic and international markets (Rahman et al., 2021).

Chilgoza plays a significant role in the socio-economical development of the rural society in Afghanistan. The natural habitat of *Pinus gerardiana* in Afghanistan, India, and Pakistan consist only of mature and over-mature trees. In contrast, seedling and young trees are limited to very harsh topography and areas covered by thorny shrubs. 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 and Pettenella, 2017). Since 2018, the trade value of Afghanistan chilgoza has improved due to an agreement between China and

Afghanistan. However, over-exploitation and grazing of livestock (sheep and goats) and inherent seed dormancy are the major concerns that pave the way for low regeneration. The species has entered the endangered groups enlisted by the IUCN (UNEP, 2008).

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 and Shamet, 2008). This important species has vanished or been exterminated due to severe biotic interference and a lack of regeneration (Kumar et al., 2014). In order to keep the species from going extinct, it is crucial to develop mechanisms for regeneration. Enhancing the germination process is essential for improving the regeneration of a species. A species should have higher germination and seedling growth for successful forest conservation.

The 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. The growing medium's composition affects the quality of the planting material, depending on other factors that may change. Soil media and gibberellic acid (GA_3) significantly affect the germination and seedling growth of different forest tree seeds (Sappalani et al., 2021). Bhardwaj et al. (1986) showed that soil media containing an equal proportion of soil, sand, and farm yard manure (FYM) was the best

medium for germination and seedling growth of *Pinus roxburghi*. Few studies revealed that GA₃ has significant effects on seed germination. A study by Amri (2010) revealed that the seed germination of *Terminalia sericea* is significantly affected by the concentration of GA₃. Similarly, Kumar et al. (2014) found a positive influence of GA₃ on seed germination of *Pinus gerardiana*.

Chilgoza pine is a very slow-growing species that takes 3 to 4 years to reach a size suitable for planting (Luna, 2008). Consequently, there is an urgent need for the study to accelerate the process of reaching a size that can be planted and the nursery manufacture of high-quality planting materials conditions. The growth medium and GA₃ may interplay in their impact on germination and early growth of *Pinus gerardiana* seedlings. However, no such attempt has been made to assess its influence on Chilgoza pine. Therefore, this investigation was conducted to determine the effects of various sowing media and GA₃ concentrations on the germination and growth aspects of Chilgoza pine seedlings. The present findings can assist Chilgoza nursery growers in enhancing the seeds' germinating potential and accelerating their nursery production cycle.

2. Materials and Methods

Study area

Paktia province of Afghanistan lies between 33.6° N and 69.5° E (Fig. 1). Paktia is a predominantly hilly province where most residents reside in the central valley, stretching from Ahmadkhel in the east down

through Zurmat and into the adjacent province of Paktika. Tsamkani and Dand Aw Patan are two valleys that lead into Pakistan in the province's eastern region. Paktia, an Afghan province in the southeast, only makes up 0.9% of the country's total land area, with a total size of 5583.2 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. With Chilgoza pine (*Pinus gerardiana*), holly oak (*Quercus baloot*), plants of the beech family (Fagaceae), and cedar, the forest is drier between 2,100 and 2,500 meters in height (Cedrus). The monsoon rains increase between 2,500 and 3,100 meters, where more deciduous trees can be found amid the conifers. The Morinda spruce (*Picea smithiana*), Bhutan pine (*Pinus*

wallichiana), *Quercus semecarpifolia*, and Himalayan cedar grow in this potentially dense woodland (*Cedrus deodara*). 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 8.0 to 8.5. Tillage and nutrients are well-tolerated by these soils.

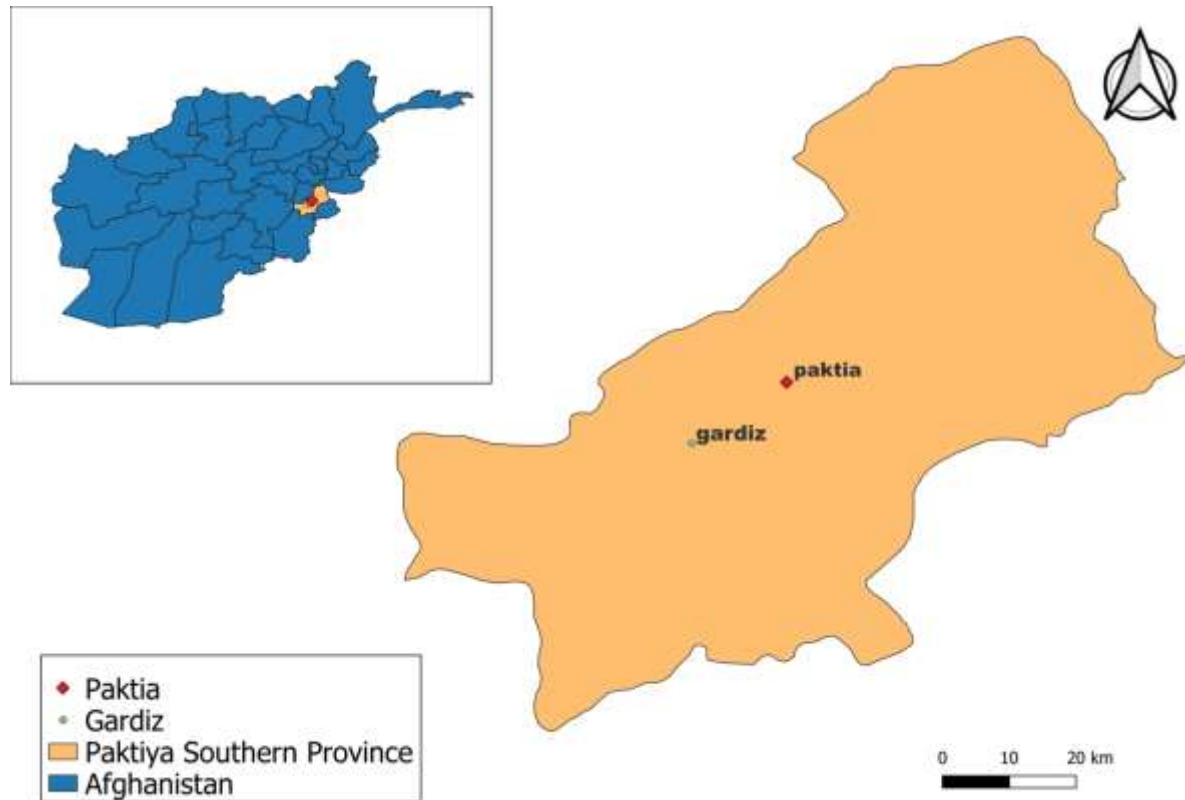


Fig. 1 Map of the study area

Experimental design and treatment details

The present study was conducted in 2018 from March to August at the Agriculture research farm of Paktia university, located at 33°38'53" N and 69°13'58" E of Gardiz city (Fig. 1). The experiment was carried out in a completely randomized design (CRD) with two factors, three replications per treatment and 50 seeds per replication, and was applied to the chilgoza pine (*Pinus gerardiana* Wall.). The seeds were obtained from local people near the chilgoza forest in Paktia province, and one-gram sachets of GA₃ were obtained from the local market, available in powder form. For the preparation of different concentrations of GA₃ (control- 0 ppm, 50 ppm, and 100 ppm), initially, the considered amount was dissolved in 1 ml⁻¹ ethanol; then, by adding distilled water, the volume was raised to determine concentrations. In the case of soil media, we used soil, sand, and FYM in different volume proportions, viz., M₁ (1:1:1), M₂ (1:1/2:1/2), and M₃ (1:1/3:2/3). Firstly, the seeds were treated for six hours with various concentrations of GA₃ and left to dry. Afterward, the seeds were quickly immersed in a diluted TERM (commercial name of fungicide) solution (0.5ml L⁻¹). The seeds were then sown in different media polybags and placed in a greenhouse. Irrigation was given manually on a weekly basis through a water sprayer. Manual weeding was done when needed.

Data collection and analysis

The day to the first germination was recorded. After then, the number of germinating seeds was recorded daily. The

28th and 68th days were indicated as first and final germination days, respectively, before and after which additional germination did not happen. For measurement of weight, seedlings were carefully removed from polybags. Firstly, the seedling length was measured through a common ruler from the crown to the topmost point, then weighed for fresh weight by a digital scale. Seedlings were transferred to an oven and were kept at 70°C for 72 hours. After drying, the dry weight of the seedlings was measured. Some parameters were estimated according to the following formulas:

Final Germination Percentage (FGP)

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

$$FGP = \frac{\sum GS}{\sum SS} \times 100$$

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).

Mean Germination Time (MGT)

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

$$MGT = \frac{\sum F * X}{\sum F}$$

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;

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

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

Germination Rate Index (GRI)

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

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

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.

Seedling Vigor Index (SVI)

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

$$SVI = SDW \times FGP$$

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

Statistical analysis

Data were processed and analyzed for ANOVA and DMRT using SAS[®] (Version#9) to test significance at 5% and mean comparisons.

Results

Sowing media, GA₃, significantly influenced FGP and their interaction (Table 1). The M₁

and G₂ treatments resulted in 55.33% and 51.55% of FGP, respectively. In contrast, the interaction of M₁ × G₂ caused 58.66% FGP (Table 1). The LSD test showed statistically significant differences within and between media, GA₃, and interaction effects (Fig. 2). Simple effects showed that the highest concentration of GA₃ (100 ppm) was not as effective as soil media (M₁) in enhancing germination. However, the interaction showed that GA₃ and media could improve germination (Table 1). As depicted in Table 1, with a gradual increase in GA₃ treatments from 0 ppm to 100 ppm, the germination percentage gradually increased from 40.22 to 51.55%, showing a ~22% increase. The final germination percentage response trend versus GA₃ concentrations was significantly positive linear and quadratic. Data analysis showed that media and GA₃ treatments significantly affected MGT (Table 2). Interaction of media and GA₃ did not significantly affect MGT. M₁ treatment caused the lowest MGT (42.36 days), and M₂ treatment had the highest MGT (45.25 days), showing a 6.5% reduction in mean germination time. The difference between M₁ and M₂ was statistically different (Fig. 3). In the case of GA₃, the shortest mean germination time (42.38 days) was obtained by G₁ (50 ppm-GA₃), and the longest mean germination time (45.16 days) was resulted by G₂ (100 ppm- GA₃) which shows approximately 6.5% reduction (Table 2). Significant differences between G₁ and G₂ treatments were significant, but between G₀ (0 ppm-GA₃) and G₂ (100 ppm- GA₃) were found to be at par (Table 2). MGT displayed a

significant quadratic response to gibberellic acid concentrations.

Table 1 Final germination percentage as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G ₁ (50 ppm)	GA ₂ (100 ppm)	
M ₁	57.33 ^{ab}	50.00 ^c	58.66 ^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		<i>LSD</i> (0.05)	
GA ₃	<i>P</i> <0.0000		2.98	
Media	<i>P</i> <0.0000		2.98	
GA ₃ ×Media	<i>P</i> <0.0000		5.17	
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.0000		Quadratic	<i>P</i> <0.0178

Table 2 Mean germination time as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	
	<i>Significance</i>		<i>LSD</i> (0.05)	
GA ₃	<i>P</i> <0.0080		1.6395	
Media	<i>P</i> <0.0063		1.6395	
GA ₃ ×Media	<i>P</i> <0.1700		2.8397	
<i>Trend vs GA₃ treatments</i>				
Linear	<i>P</i> <0.2173		Quadratic	<i>P</i> <0.0036

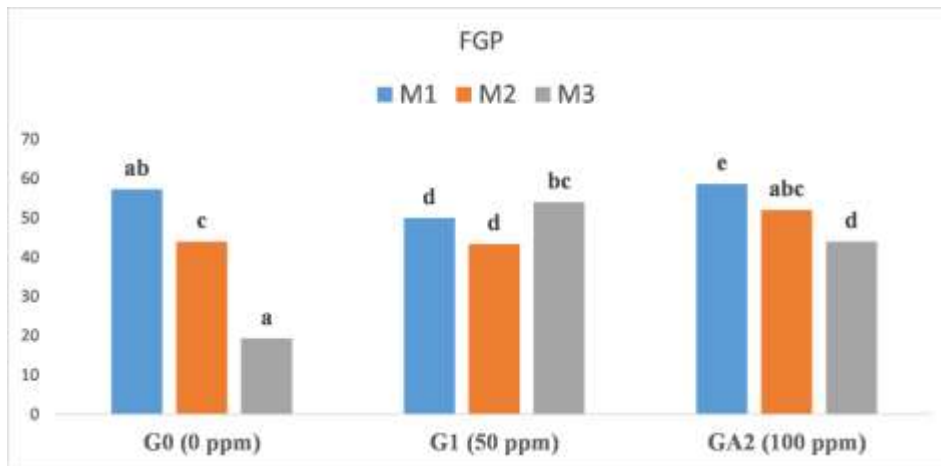


Fig. 2 Effect of gibberellic acid and planting media on final germination percentage (FGP)

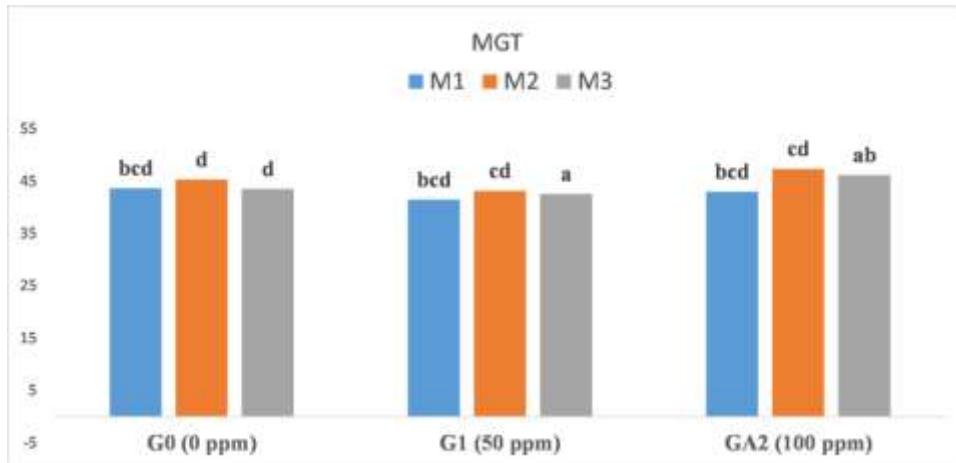


Fig. 3 Effect of gibberellic acid and planting media on mean germination time (MGT)

The variance analysis showed that the interaction between the soil medium and GA₃ substantially impacted GI (Fig. 4). The highest GI (250.889) and lowest GI (102.88) came from seeds grown in M₁ and M₂ soil media, which differed significantly (Table 3). In addition, the pre-treating seeds with 100 ppm GA₃ (G₂) followed by sowing in M₁ resulted in the highest GI (274). While, seeds sown in M₃ without any GA₃ pre-treatment (0 ppm) led to the lowest GI (56.67) (Table 3). GA₃ concentrations and GI had a non-significant linear or quadratic connection.

The media, GA₃, and interactions between the media and GA₃ were found to have a considerable impact on the seed germination index. Media treatments (M₁ and M₃)

resulted in maximum (70.19) and minimum (40.90) seed germination index with significant differences (Table 4). GA₃ at 50 ppm concentration (G₁) had the highest GRI (58.07), and G₀ (0 ppm GA₃) recorded the lowest (43.64) with significant variations as compared to control, showing a 24.84% increment in GRI (Table 4). The highest (75.01) and lowest (19.86) GRIs were obtained by the interaction of G₂ (100 ppm-GA₃) with seeding in M₁ and G₀ (0 ppm-GA₃) with sowing in M₃. As depicted in Table 4, the M₁ treatment appeared to be more effective in combining G₂ with M₁ but was more effective in enhancing GRI than G₁. Furthermore, GRI showed significant quadratic and positive linear response vs. GA₃ concentrations (Fig. 5).

Table 3 Germination index as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	
Media	<i>P</i> <0.0000		31.705	
GA ₃ ×Media	<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, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	
Media	<i>P</i> <0.0000		4.9111	

GA₃×Media P<0.0000 8.5063

Trend vs GA₃ treatments

Linear P<0.0016 Quadratic P<0.0001

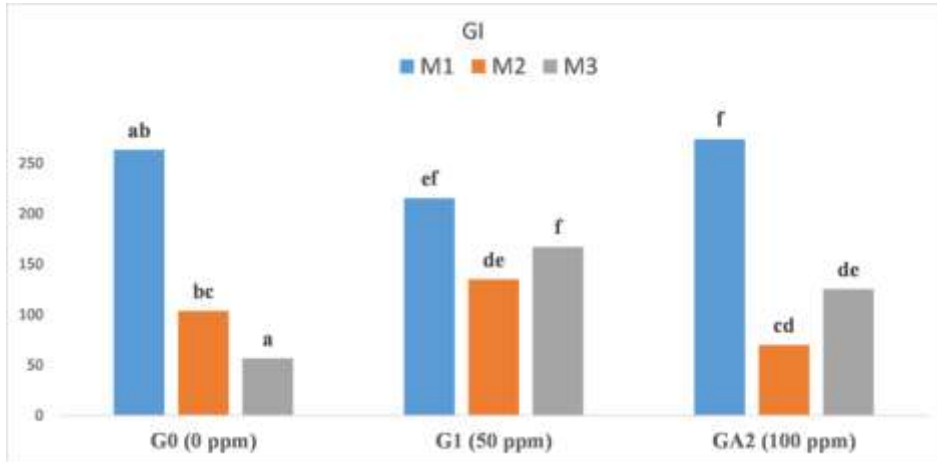


Fig. 4 Effect of gibberellic acid and planting media on germination index (GI)

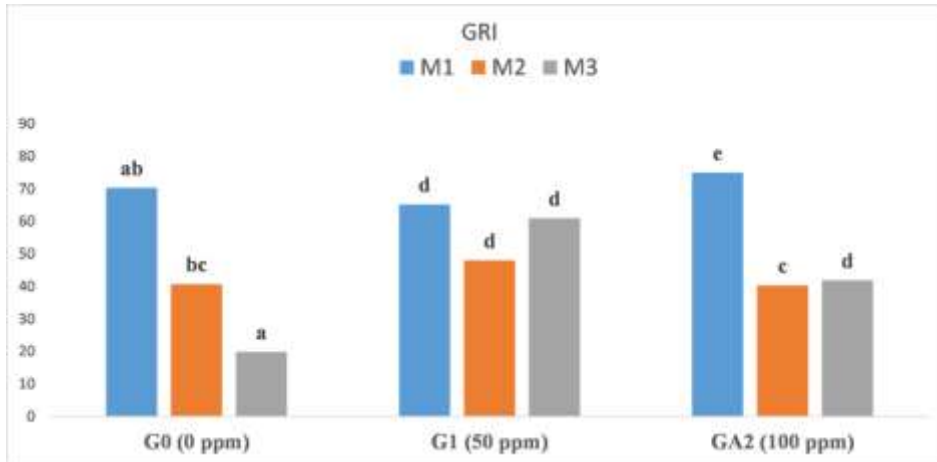


Fig. 5 Effect of gibberellic acid and planting media on germination rate index (GRI)

The GA₃, media, and interaction of media × GA₃ were found to significantly influence the fresh weight of the seedling. Maximum SFW (7.98 g) and minimum SFW (6.37 g) were obtained by M₁ and M₃ but were in the case of GA₃- G₁ (8.25 g) and G₀ (5.33 g), respectively. M₁ differed significantly with M₂ and M₃, and G₁ differed significantly with G₀ (Table 5). G₁ treatment caused a 35.39% increment in fresh seedling weight compared to G₀. Among the interaction effects, the highest SFW (8.92 g) was obtained from treating seeds with G₁ and sowing in the M₃ medium. There was a significant positive linear relationship between fresh seedling weight and GA₃ concentrations (Table 5).

Data in Table 6 shows that the various media treatments did not influence seedling dry weight, but the GA₃ treatments and their interaction with media significantly affected the SDW. Though G₂ produced the highest SDW (3.95 g), it was not significantly different from G₁. The minimum SDW (2.46 g) was recorded in G₀, which showed an approximately 38% increase (Table 6). Interactions showed seeds initially treated with G₂ and sown in M₂ had the maximum SDW (4.78 g). In contrast, the seeds which were not treated with GA₃ (G₀) and sown in the M₃ medium had the lowest (1.022g) seedling dry weight (Table 6). Seedling dry weight and GA₃ concentrations correlated positively.

As presented in Table 7, various media and GA₃ treatments and their interaction significantly influenced seedling length. In the case of media, the highest (203.85 cm) and lowest (135.72 cm) seedling lengths were recorded on M₁ and M₃ treatments, respectively. But in the GA₃ treatments, the maximum seed length (189.46 cm) and minimum seedling length (140.04 cm) were produced respectively by G₂ and G₀. G₂ increased seedling length by 26% compared to G₀. The interaction effects revealed that when seeds were treated with G₂ and sown in M₁, the highest seedling length was 219.80 cm. In contrast, the seeds which were not treated and sown in M₃ recorded the lowest seedling length (64.80 cm) (Table 7). A significant positive linear correlation was observed between seedling length and GA₃ concentrations (Table 7).

The main effects of soil media and GA₃ and their interactions considerably impacted SVI. Maximum SVI (113647.889) was recorded in M₁ and minimum (61009.378) in M₃, having significant differences (Table 8). GA₃ caused maximum SVI (99703.4) by G₂ and minimum (65636.387) by G₀, which shows about a 34% increase in SVI as compared to G₀ (control) treatment. Interaction effects showed that G₂ × M₁ and G₀ × M₃ led to the highest (129192.60) and lowest (13008.53) SVIs, respectively (Table 8).

Table 5 Seedling fresh weight as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	
Media	<i>P</i> <0.0409		1.3310	
GA ₃ ×Media	<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, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	

Media	$P < 0.1328$	0.9290
GA ₃ × Media	$P < 0.0170$	1.6090

Trend vs GA₃ treatments

Linear	$P < 0.0038$	Quadratic	$P < 0.7552$
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Table 7 Seedling length as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	$P < 0.0000$		15.014	
Media	$P < 0.0000$		15.014	
GA ₃ × Media	$P < 0.0000$		26.006	

Trend vs GA₃ treatments

Linear	$P < 0.0000$	Quadratic	$P < 0.2566$
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Table 8 Seedling vigor index as affected by gibberellic acid, planting media and their interaction

Planting Media	Gibberellic acid			Means for Media
	G ₀ (0 ppm)	G 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	P<0.0001		12038	
Media	P<0.0000		12038	
GA ₃ ×Media	P<0.0000		20851	
<i>Trend vs GA₃ treatments</i>				
Linear	P<0.0000		Quadratic	P<0.5628

3 Discussion

This study showed that various media, GA₃, and their interaction treatments significantly influenced the germination percentage of chilgoza pine seeds. However, the effect of the media was more prominent, where the medium of soil, sand, and FYM with equal ratios resulted in the highest germination. In contrast, germination percentage gradually increased with increasing GA₃ concentrations; that is, 100 ppm GA₃ increased FGP ~22% compared to 0 ppm GA₃. The growth medium provides aeration and nutrition conditions, and GA₃ promotes activities of hydrolyzing enzymes responsible for starch degradation leading to enhancement in the germination of seeds (Nile et al., 2022). Our findings are in line with those reported by Galston and Davis (1969), Verma and Tandon (1988), and Bahardwaj et al. (1986).

The 50 ppm- GA₃ and M₁ treatments had equal importance in reducing mean germination time, but 100 ppm- GA₃ delayed germination time as the control (0 ppm- GA₃) treatment. This delay might be

due to a higher concentration of GA₃. Our results showed that GA₃ alone was ineffective in the seed germination index, but sowing media resulted in the seed germination index. This indicates that GA₃ is essential during the early stages of germination, while after dormancy has broken, growing media become necessary for later germination stages like radicle protrusion and emergencies. The study showed that GA₃ treatment substantially increased the fresh weight of seedlings than media treatments and interactions. This increase might be due to cell enlargement and turgor pressure events stimulated by GA₃ (Sprangers et al., 2020). Similar findings have been reported by Sudhakar et al. (1995) and Lavania et al. (2006).

A positive linear relationship existed between GA₃ concentrations and FGP, GRI, SFW, SDW, SL, and SVI, which means the higher the GA₃ concentration, the higher the correlated parameters. Positive quadratic trends were seen only in the case of FGP and MGT. Although this issue presents a general response image of different parameters vs. GA₃ concentrations, in future studies, it is necessary to be included more

intervals of GA₃ concentrations with narrower ranges to increase the accuracy and precision of the response.

4. Conclusion

Findings reveal that the sowing media and GA₃ and their interaction significantly affected seed germination and seedlings' growth. The best treatment was the interaction of 100 ppm- GA₃ and M₁. The main effects of media were better than GA₃ on seed germination, while simple effects of GA₃ resulted in higher vegetative growth of seedlings. All the studied parameters responded linearly against GA₃ levels, but the trend was quadratic in the case of FGP and MGT. For further investigation, it is recommended to use various GA₃ levels on vegetative growth of seedlings either as ground or foliar application.

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Declarations

Conflict of interest

All authors declare that they have no competing interests.

Ethical standards

For this article no studies with human participants or animals were performed by any of the authors. All studies mentioned were in accordance with the ethical standards indicated in each case. For images or other information within the manuscript which identify patients, consent was obtained from them and/or their legal guardians.

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