

Effect of stratification and dipping with different concentrations of gibberellic acid in the vegetative and root growth characteristics of *Pinus eldarica* Medw.

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Received:	Abstract
Oct. 15, 2021	This study was conducted on the seeds of <i>Pinus eldarica</i> during the
	period from February 2020 to September 2021 to find out the effect
	of cold stratification at (°4C) for (60 days) and different concentra-
Accepted:	tions of gibberellic acid (0, 200,400 mg. L-1) in the traits of the veg-
-	etative and root growing seedlings. The results showed the superior-
Nov. 04, 2021	ity of the stratified seeds for the characteristics (seedling height, stem
	diameter, number of branches, number of leaves, root length, dry
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r ublisheu:	results of dipping in gibberellic acid showed a superior concentration
Dec. 01, 2021	(400 mg.L-1) for traits (seedlings height, leaves number, length of
	the main root, dry weight of the shoot, dry weight of the root system).
	The concentration of 200 mg.L-1 for the two traits (stem diametet,
	branches number) was superior. The results of the binary interaction
	of stratification and gibberellic acid showed superiority of the strati-
	fied seeds with two concentrations (400 mg.L-1 and 200 mg.L-1) for
	traits (seedling height, eaves number, length of main root, dry weight
	of shoot, dry weight of root) and (Stems diameter Branches number)
	respectively.
	Keywords : <i>Pinus eldarica</i> , vegetative growth, root growth

Introduction

Pinus eldarica, is one of the most important species of the Pinus genus. It is a tolerance and fast-growing species compared with other species of Pinus genus and tolerant of drought and both of the high and low temperatures as well [1]. Therefore, many countries have sought to cultivate and raise it for the purposes of afforestation of arid areas and combating desertification and as windbreaks and afforestation in parks, roads and public squares, and it is raised as Christmas trees [2]. The method of propagation by seeds is the most common method to propagation of conifers in general as it is the easiest and cheapest, and is used as the main means of propagation of *Pinus eldarica* [3].

One of the problems of propagation by seeds is the dormancy phase as a result of various genetic and environmental factors and for the purpose of breaking dormancy in seeds and stimulation. the growth process of seedlings, many physical and chemical methods have been used in order to stimulate the embryo and break the dormancy phase. the most important of which is the method of stratification. Temperature and duration



of stratification have a significant effect in seed germination and subsequent growth of seedlings [4] stratification in low temperatures between (1-10)°C, breaks Seeds dormancy which breaks down in hibitors and lead to the formation of gibberellic acid GA3, which increases the concentration of starch hydrolysis enzymes and turns it into digestible and oxidative sugars, which provides Energy required for stimulation and physiological activity of the fetus [5]. It was also indicated [6] that the effect of the periods and conditions of stratification and dipping in hot water for Melia azedarach L. seeds gave the best rates for the characteristics of the seeds and the subsequent vegetative growth of the seedlings, They found that the results of the stratification period in (45) days gave the best rates for the studied trait (germination percentage, vegetative energy, seedling length, seedling diameter, and leaves number) compared with the hot water immersion treatment that gave lower rates for the studied traits. It was showed [7] in a study on the effect of cold stratification on the percentage of seed germination and growth of seedlings of Ziziphus lotus L. The Sidr seeds were stratified for periods (0,45,90,120) days at a temperature of $(5^{\circ}C)$, the stratification period of 120 days was significantly superior, giving the highest germination rate of 83% and the highest stem height of 16.5 cm and the highest root length of 9.75 mm compared with the division periods.

It is possible to speed up the process of seed germination by accompanying some growth regulators with stratification one of them gibberellic acid [8]. Gibberellic acid is one of the most important plant hormones that are naturally produced in new leaves and germinated seed embryos [9] its work cell division and elongation which increases vegetative growth, especially longitudinal growth [10]. Additionally, it was indicated [11] the effect of treatment with different concentrations of GA3 and with different immersion periods in the germination and growth of canary pine seedlings, three concentrations of gibberellic acid were used, 0, 200 and 300 ppm, and for different immersion periods (0, 10 and 15). The results showed that the concentration of 300 ppm of gibberellic acid was superior to all studied traits (germination percentage, survival rate, length of seedlings, dry weight of stem, leaves and roots), the immersion period of 20 minutes showed a significant superiority in its effect in seedling length, weight dry, the stem, leaves and roots over the rest of the immersion periods. The results of the interaction showed the superiority of the seeds immersed in a concentration of 300 ppm of gibberellic acid and for a period of 20 minutes was a significant superiority in the dry weight of stems and leaves. It was mentioned [12] that the best height of pistachio seedlings and the diameter of the main stem was obtained by soaking the seeds in gibberellic acid at a concentration of 200 mg. L-1 for 12 hours.

The aims of this research to obtain the best germination of seeds and the fastest growth of seedlings and the best characteristics of their vegetative and root system by treatment *P. eldarica* Medw seeds by stratification and immersion with different concentrations of gibberellic acid.

Materials and methods



Pinus eldarica seeds were used in this study, brought from Diyarbakir / Turkey with a high purity of 99% and a vitality of 83%. The Seeds treatment before planting by:

A - Cold stratification of a section of seeds at (4-5) ° C for a 60 days.

B - Treatment of stratified and non stratified seeds with different concentrations of gibberellic acid (GA3) (0, 200 and 400 mg. L-1) for (24 hours).

The seeds planted in the forest department nursery in 10/2/2020 and the results were taken after the seedlings grew on 30/9/2020. The average traits were taken from five seedlings that were randomly selected from the experimental units.

1-Seedlings height (cm. seedling⁻¹): The readings were taken using a tape measure from the surface of the soil of the bag to the top of the plant and for all seedlings.

2- Stem diameter (mm. seedling⁻¹): The diameter was measured at a height of one centimeter from the surface of the soil of the bag and for all seedlings by means of a digital foot (Vernier).

3- Leaves number (leaf. seedling⁻¹): The total number of leaves for a selected sample of the largest seedlings was calculated in each experimental unit for each treatment.

4- Branches number. (branche. Seedlings⁻¹): The number of branches was counted, except for the main stem .

5- Root length (cm): The root length of dracaena pine seedlings was measured using a tape measure from the point of contact of the root with the stem to the end of the growing tip of the root

6- Dry weight of the vegetative (gm. seedlings⁻¹): The shoots of five seedlings in each experimental unit were dried using an electric oven at a temperature of 70 ° C \pm 1 and until the weight was stable, then weighed using a sensitive electric balance with a sensitivity of 01.0 mg and extracted average

7- Dry weight of the root system (g. seedlings⁻¹): Dry the root total of five seedlings in each experimental unit after separating it from the vegetative growth and removing the dust that is stuck in it using an electric oven at a temperature of $70^{\circ}C \pm 1^{\circ}C$ until the weight is stable and then weighed using an electric oven at a temperature of $70^{\circ}C \pm 1$ A sensitive electric balance with a sensitivity of 0.1 mg and extracted its average.

Results and Discussion

Effect of stratification and immersion with different concentrations of gibberellic acid in height of seedlings of *Pinus eldarica* (cm. seedling⁻¹):-



The results of table (1) showed that stratification was significantly superior to the height of *Pinus eldarica* Seedlings growing from stratified seeds as the average height of seedlings reached (24.77 cm) while the average height of seedlings grown from seeds that were not stratified was 18.82 cm. The results of immersion in acid Gibber-ellic showed the superiority of the concentration (400 mg. L⁻¹) which recorded the highest average of seedling height of (26.06) cm compared with the control treatment which gave the lowest rate of seedling height of (19.06 cm). The results of the binary interaction indicate the superiority of the division Stratified with concentration (400 mg. L⁻¹) and obtained the highest average of seedling height, which amounted to (26.29) cm compared to the control treatment which gave (15.25) cm.

Gibberellic acid	The interaction effect between stratification and gibberellic acid		The effect of gib- berellic acid mg.l ⁻¹
conc. mg.l ⁻¹	Without stratification	Stratification	bereine aciu ing,i
0	15.25 f	24.77 b	19.06 c
200	18.68 e	21.97 d	19.99 b
400	22.54 с	26.29 a	26.06 a
Effect of stratification	18.82 b	24.32 a	

Table (1) Effect of typesetting and immersion with different concentrations of gibberellic acid in height of *P. eldarica* seedlings (cm. seedling-1)

2- Effect of stratification and immersion with different concentrations of gibberellic acid in the stem diameter of *Pinus eldarica* seedlings (mm. seedling⁻¹):

The results of table (2) indicate the effect of stratification on the stem diameter as the seedlings grown from the stratified seeds gave the highest average of the stem diameter characteristic (4.01 mm) while the average of the stem diameter of the seedlings grown from non-stratified seeds decreased to (2.45 mm). The results of immersion in gibberellic acid indicate the superiority of the treatment with the concentration (200 mg. L⁻¹) and recorded the highest rate of the characteristic amounted to (4.05 mm) compared with the control treatment which gave the lowest average for the diameter of the stem amounted to (2.26 mm). The results of the binary interaction of stratification with gibberellic acid to Stratified seeds and concentrated treatment (200 mg.l⁻¹) showed the highest average of the trait, which reached (6.36 mm) while the average of stem diameter decreased to (1.93 mm) in the control treatment.



Table (2) Effect of stratification and immersion in gibberellic acid with different concentrations in the stem diameter of *Pinus eldarica* seedlings (mm. seedling-1)

Acid Gibberellic conc. mg.l ⁻¹	The interaction effect between stratification and gibberellic acid		The effect of gibberellic acid mg.l ⁻¹
••••••••••••••••••••••••••••••••••••••	Without stratification	Stratification	
0	1.93f	2.77 c	2.26 с
200	2.44e	6.36 a	4.05 a
400	2.99 b	2.69 d	2.88 b
Effect of stratification	2.45b	4.01 a	

3- Effect of stratification and immersion with different concentrations of gibberellic acid in the number of branches of *Pinus eldarica* seedlings (branch. seedling⁻¹):-

The results of table 3 show the effect of stratification significantly on the characteristic of the number of branches in the seedlings of *Pinus eldarica*, as the seedlings grown from the stratified seeds gave the highest rate of the character reached (8.16 branches. seedling ⁻¹) While the average number of branches for seedlings growing from seeds that are not stratified was (6,05 branches. seedling⁻¹). and the results of immersion in gibberellic acid indicate the superiority of the seeds treated with concentration (200 mg. L⁻¹) which recorded the highest rate for the trait amounted to (9.58 branch. seedling⁻¹) compared with the control treatment, which gave the lowest rate of number The branches reached (6.48 branche. seedlings⁻¹). The results of the binary interaction of typesetting and gibberellic acid indicate the superiority of the stratified seeds treated with concentration (200 mg.L⁻¹) in obtaining the highest average for the trait, which reached (14.90 branches.seedling⁻¹) compared to the control treatment that gave the lowest average. For the adjective (5.40 branches. Seedling⁻¹).

Table (3) Effect of stratification and immersion with different concentrations of gibberellic acid in the number of branches of pine seedlings *P. eldarica* (branch. seedling-1):-

Acid Gibberellic	The interaction effect betwee and gibberellic	The effect of gibberellic acid	
conce. mg.l ⁻¹	Without stratification	Stratification	mg.l ⁻¹
0	5. 40d	10. 94b	6.84c
200	5. 81d	14. 90a	9.58a
400	6. 94c	11. 53b	7.71 b
Effect of stratification	6.05b	8.16 a	,



4- Effect of stratification and immersion with different concentrations of gibberellic acid in the number of leaves of *Pinus eldarica* seedlings (leaf. seedling⁻¹):-

The results of table (4) indicate the effect of stratification in the characteristic of the number of leaves of *Pinus eldarica* seedlings, as the seedlings grown from the stratified seeds gave the highest average of the trait reached (949.8 leaves. Seedling⁻¹) while the average number of leaves for seedlings grown from the seeds The non stratified (740.727 leaves. seedlings⁻¹). The results of immersion in gibberellic acid indicate the superiority of the concentration (400 mg. L⁻¹) significantly which recorded the highest average of the trait reached (960.69 leaves. seedlings⁻¹). Compared with the control treatment, which gave the lowest average number of leaves amounted to (733.7 leaves. seedling⁻¹). The results of stratification and gibberellic acid indicate the superiority of the stratified seeds treated with concentration (400 mg. L⁻¹) significantly in obtaining the highest rate. for the phenotype, which amounted to (1032 leaves. seedling⁻¹) compared with the control treatment, which gave the lowest average for the phenotype (560,60 leaves. sappling⁻¹).

Acid Gibberellic conce. mg.l ⁻¹	The interaction effect between stratification and gibberellic acid		The effect of gibberellic acid
conce. mg.i	Without stratification	Stratification	mg.l ⁻¹
0	560.60f	991.79b	733.07с
200	740.73e	836.73d	779.21b
400	920.84c	1032a	960.69a
Effect of stratification	740.727b	949.08a	

Table (4) Effect of stratification and immersion with different concentrations of gibberellic acid in the number of leaves of *P. eldarica* seedlings (leaf. seedling-1)

5- Effect of stratification and immersion with different concentrations of gibberellic acid with different concentrations in the main root length of *Pinus eldarica* seedlings (mm. seedling⁻¹):-

It is noted from table (5) the significant effect of stratification in the main root length of *Pinus eldarica* seedlings, as the highest average root length in seedlings grown from stratified seeds reached (26.23 cm) while the average root length of seedlings grown from non-stratified seeds reached to 22.04 cm. The results of immersion in gibberellic acid indicate the superiority of concentration (400 mg.L⁻¹) which recorded the highest average of root length of (18.27) cm compared with the control treatment. The results of the binary interaction of stratification and gibberellic acid indicate that the stratified seeds and treated with concentration (400 mg. L⁻¹) were significantly superior, and the



highest average of root length was (27.63) cm compared to the control treatment that gave (19.63) cm.

Table (5) Effect of stratification and immersion with different concentrations of gibberellic acid with different concentrations in the main root length of *P. eldarica* seedlings (mm. seedling⁻¹)

Acid Gibberellic conce. mg.l ⁻¹	The interaction effect between stratification and gibberellic acid		The effect of gibberellic
	Without stratifica-	Stratification	acid mg.l ⁻¹
0	19.63f	27.54b	21.18 c
200	23.37 d	23.77c	22.59 b
400	22.85 e	27.63a	27.18 a
Effect of stratification	22.04 b	26.23 a	

6-The effect of stratification and immersion with different concentrations of gibberellic acid in the dry weight of the vegetative of *P. eldarica* seedlings (gm. seedling-1)

The results of table (6) show the effect of stratification significantly in the dry weight of the vegetative growth of *Pinus eldarica* seedlings, as the highest average of the trait in seedlings grown from the stratified seeds was (3.26 g), while the average dry weight of the vegetative growth in the growing seedlings From seed non stratified 2.54 g. The results of immersion in gibberellic acid indicated that the concentration (400 mg.L⁻¹) was significantly superior, and the highest average for the character was (3,34) g compared with the control treatment, which gave less for the character reached (2,53 g). The results of the binary interaction of stratification and gibberellic acid indicate the superiority of the stratified seeds and treated with concentration (400 mg.L⁻¹) and recorded the highest average for the trait, which amounted to (3.75)gm compared to the control treatment that gave the lowest rate for the trait (2, 16) grams.

Table (6) The effect of stratification and immersion with different concentrations of gibberellic acid in the dry weight of the vegetative of Pinus eldarica seedlings (gm. seedling-1)

Acid Gibberellic	The interaction effect between stratification and gibberellic acid		The effect of
conce. mg.l ⁻¹	Without stratification	Stratification	gibberellic acid mg.l ⁻¹
0	2.167e	3.01c	2.53 c
200	2.35d	3.10b	2.65b
400	3.11b	3.75 a	3.34a
Effect of stratification	2.54b	3.26a	



7- Effect of stratification and immersion in gibberellic acid at different concentrations. Dry weight of the root growth of *Pinus eldarica* seedlings (gm. seedling⁻¹)

The results of Table (7) indicate the effect of moral stratification on the dry weight characteristic of the root growth, as the seedlings grown from the stratified seeds of Pinus eldarica gave the highest average of the trait amounted to (1.95 g) while the average dry weight of the root growth of seedlings grown from Stratified seeds(1.47)g and the results of immersion in gibberellic acid indicate a superior concentration (400 mg. L⁻¹) which recorded the highest rate for the character amounted to (1.70) g compared with the control treatment, which gave less for the characteristic amounted to (1.58 gm). The results of the binary interaction of the stratification and gibberellic acid indicate that the stratified seeds treated with concentration (400 mg. L⁻¹) were significantly superior in obtaining the highest average dry weight of the root growth, which amounted to (2.12) g in comparison with the control treatment that gave (1.40) g.

Table (7) Effect of stratification and immersion in gibberellic acid at different
concentrations. Dry weight of the root growth of P. eldarica seedlings (gm. seed-
ling-1)

Acid Gibberellic conce. mg.l ⁻¹	The interaction effect between stratification and gibberellic acid		The effect of gibberellic acid
conce, mg.	Without stratification	Stratification	mg.l ⁻¹
0	1.40f	1.84c	1.58 c
200	1.53d	1.92b	1.68 b
400	1.47e	2.12a	1.707a
Effect of stratification	1.47b	1.95a	

It is noted from the results of tables (1-7) that the moral effect of stratification in all the studied traits may be due to the fact that the degree and duration of cold stratification has a significant effect on seed germination, and this effect is by stimulating the dormant fetus to activity gradually as a result of some physiological changes in it [4]. The reason may be that the low temperatures in the stratification process, which range between (1-10)^o C lead to breaking the dormancy of the seeds which works to break down inhibitors and lead to the formation of gibberellic acid GA3 to an increase in the concentration of starch hydrolysis enzymes and its transformation into indigestible sugars. and oxidation, which provides the energy needed to stimulate growth and the physiological and vital activity of the growth process [5]. The different concentrations of gibberellic acid significantly affected the height of seedlings, the number of leaves and branches, and the reason may be due to the fact that the process of seed germination



requires an effective enzymatic system to carry out the building and demolition processes during the germination [13]. the high concentration of gibberellic acid plays a positive role in stimulating the embryo to germinate, and accelerates the growth of the vegetative and root system of conifers and slow-growing species, as gibberellic acid reduces the inhibitory role of abscisic acid and encourages the construction of hydrolysis enzymes and increases their effectiveness, and this is consistent with what it was found previously [14]. In the fact that increasing the periods of immersion in gibberellic acid helps to improve the process of seed germination and the formation of an advanced root growth and encourages vegetative growth and reduces the effect of growth inhibitors.

References

- 1) Dickison, W.C. (2000). Integrative plant anatomy. Academic Press, San Diego, USA.
- 2) Rudall, P. J. (2007). Anatomy of flowering plants an introduction to structure and development. Cambridge University Press, New York, USA.
- 3) Jericó, B.B.; Lourdes, I.A.; Lázaro, S.V.; José, C.M. and Nancy, S.B. (2012). In vitro regeneration of Pinus brutia Ten. var. eldarica through organogenesis. African Journal of Biotechnology. 11(93): 15982-15987.
- **4**) Luna, B.; Pérez, B. ; Torres, I. and Moreno, M.J. (2012). Effects of incubation temperature on seed germination of mediterranean plants with different geographical distribution ranges: folia geobotanica journal of the institute of botany, academy of sciences of the Czech Republic 47 (1).
- **5)** Bewley, J.D. (2007). Seed Germination and Dormancy. American Society of Plant Physiologists, Department of Botany, University of Guelph, OntarioNIG2W, Canada.9.1055-1066.
- 6) AI- Khafaf, R. S. and Sh. L. Yaqoub (2013). The effect of periods and conditions of stratification and immersion in hot water on the germination of Melia azedarach L. seeds and the growth of its seedlings: Al-Rafidain Agriculture Journal 41 (1).
- 7) Kheloufi, L.M. and Mansouri, K. L. (2020). Effect of cold stratification on seed germination of the multipurpose fruit shrub, ziziphus lotus (L.) LAM. (Rhamnaceae) A. : Cercetări Agronomice în Moldova. 3(2) 152-159.
- 8) Donald. E. R; Kathryn, E. K.; Tahar, A-A. and Nicholas, P. H. (2001). How Gibberellin regulates plant growth and development: A Molecular Genetic Analysis of Gibberellin Signaling, Annual Review of Plant Physiology and Plant Molecular Biology 52:67-88.



- 9) Al-Rawi, A. G. S. (2011). Response of mungbean (Vigna radiata L., Wilczek) to gibberellic acid (GA3) rates and varying irrigation frequencies. Int. J. Biosci. (1) 85–92.
- Taiz, L. and Zeiger, E. (2010). Gibberellins: regulators of plant height, in Plant Physiology, ed E. Zeiger (Sunderland, MA: Sinauer Associates, Incorporated), 461–493.
- **11**) Al-Hadidi, S. H. A. (2005). Effect of concentrations and periods of immersion in gibberellic acid on seed germination and growth of canary pine seedlings, Mesopotamia Journal of Agriculture 32 (4).
- **12**) Al-Imam, N. M. A. A. (2007). The effect of soaking periods, gibberellic acid and benzyl adenine on the germination of pistachio seeds and the subsequent growth of seedlings (*Pistacia vera* L.), Mesopotamia Journal of Agriculture 35 (2).
- **13**) Attia, H. J; M. Shurooq, K. S. and Bashir, A. I. (2010). the effect of plan growth regulators on some vegetative characteristics of the black seed, College of Agriculture, University of Baghdad 41 (2): 80-88.
- 14) Al-Asho, J. A. and Riyadh, S. Kh. (2000). The effect of seed weight and gibberellic acid on the germination and growth of fruiting pine seedlings, Al-Rafidain Agriculture Journal, 23 (1): 61-65.