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Research Paper

### Improved characterization of seed storage *pinus radiata* with gibberellic acid (GA<sub>3</sub>) and potassium nitrate (KNO<sub>3</sub>)

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#### Abstract

Improved characterization of fresh seeds to increase crop yield achieved in many cases by using priming and plant growth regulators have been carried out. But sometimes the seeds in storage and during this period, depending on storage conditions, have gradually become deteriorated. In this condition, the seed quality decreases and, finally, the plant has a lower performance than other plants. This study aimed to investigate the possibility of improving germination and vegetative characteristics of storage seeds of radiata pine (*Pinus radiata*). Treatments by gibberellic acid (GA<sub>3</sub>) in concentrations of 200, 300 and 400 ppm and potassium nitrate (KNO<sub>3</sub>) in a concentration of 0.2, 0.3 and 0.4 mol/liter, soaking time for 24 and 48 hours, the two bed of sand and peat were applied. Factorial experiment was conducted in completely randomized design in four replications. Treatments on all characteristic were significant for both germination and vegetative traits. Most treatments had positive results; however, treating by gibberellic acid 300 in 48 hours and then treating by potassium nitrate 0.3 (in 24 and 48 hours) sands to improve the characteristics of both germination and vegetative characteristics were more suitable.

**Keywords:** gibberellic acid (GA<sub>3</sub>), *pinus radiata*, potassium nitrate (KNO<sub>3</sub>), seed storage

#### Introduction

For different causes, sometimes, seeds are kept in storage and during this time have become deteriorated. Depend on condition of store, gradually. Decreasing of integration of membrane, changing in molecular structure of nucleic acids and reduction in enzyme activity is the most important changes during seed deterioration (Justice and Bass, 1979). These changes resulted in decreasing quality of seed, percent and rate of germination, and, in some cases, growth and function of plant as well as rising of sensitivity to environmental stresses (Coin et al, 1995). Decreased growth causes to reduce in competing with weeds, less ghosting on the soil surface and thus reduce soil moisture through evaporation (Soltani, 2004; Soltani et al, 2001). Then poor seedling, which have less growth than normal seedling, have fewer environmental features (light, moisture and food) are more sensitive to unfair environmental condition. This difference in initial growth of plants may continue during the lifetime of the plant and effect on function of plant (Basra et al, 2003). The effects of seed deterioration can be decreased by variant ways such as priming and plant growth regulators externally, because according to experiments, external application of these compounds on seeds affects their inner surface (Qiang et al, 2005; Thomas, 1990). Priming is a method for increasing power and establishment of seedlings (Golzar et al, 2001) and it is conducted by using salting solutions (osmo-priming), matrix materials, organisms such as bacteria, fungi and alga (bio-priming), water in moving containers (drum priming) (Parera & Cantliff, 1994). Mineral salt solution like potassium nitrate, potassium phosphate, sodium chloride, magnesium soleplate or some organic materials like polyethylene glycol, mannitol, glycerol and polypropionate are used in osmo-priming for decreasing osmotic potential of water (Lotfi et al., 2011). Also, plant growth regulators play an important role in critical processes of plant. These compounds are produced in a part of plant and make some stimulating or inhibitory effects after transferring or in that part (Salisbury & Ross, 1978). Gibberellin is one of the plant hormones that have a critical role in biology and germination and shows different regulatory effects in variant processes (Kermode, 2005; Finkelstein, 2008). The release of gibberellin during seed germination and passing the cold period causes breaking sleep of seeds and sprouts. In long day plants (in aspect of optical rotation in forming flowers), gibberellin can be replaced for red photoperiod (Nasirzadeh et al., 2007). Many studies have confirmed positive effects of using priming and hormones for improving equality of seed. Larionova (1997) found gibberellin increases the germination rate, stimulates seedling growth and decreases variety in sizes of *Larix sibirica*, *Pinus sylvestris* and *Picea obovata*. Biswas et al. (1972) studied the effect of stratification, potassium nitrate and gibberellins on increasing germination in *Pinus taeda* and *Taxodium* seeds. They found, although, all of three treatments have better results than control, but treatment by gibberellin, for 21 days and in a concentration of 100 mg/Lit, has the best effete. Lavania et al. (2006) evaluated the effect of gibberellic acid on germination of *pinus wallichiana* and expressed the highest germination had been occurred in concentration of 300 ppm during 24 hours and 200 ppm in 36 hours and the concentration may be decreased by raising time of suspension in hormone. Little and MacDonald (2003) investigated the effect of gibberellin on elongation of stem and vegetative extension of *Pinus sylvestris* and *Picea glauca* saplings found that gibberellin causes to increase in length of stem. The results of evaluation of different lights on seed germination in pins showed KNO<sub>3</sub> and light had a same effect on seeds in dark. But it had no effect on seeds in normal condition (Sasaki & Asakawa, 1974). In a study on seed germination in Scots pine, it was reported treatment of seeds with KNO<sub>3</sub>, in concentration of 0.02 and 0.05 M, stimulates germination. Also, the energy of germination increases in concentration of 0.05 M, significantly, but the energy and capacity of germination decreases in higher concentration (1 and 2 M) (Barzdajn, 1986). In another investigation by Ghildiyal et al. (2009), soaking seeds of *Pinus roxburghi* in 10 mg/Lit of GA<sub>3</sub> increased the germination 8 % more than the control and it reduced the germination period as much as 8 to 10 days. This research was

conducted to evaluate the improvement of seedling germination and vegetation properties of stored seeds in *Pinus radiata* (Provenance new Zealand) that were kept in center of seeds located in Amole, Koloudeh.

### Materials and Methods

The seeds of *Pinus radiata* with the origin of New Zealand (collected in 1990) were prepared from the Caspian Forest Seed Centre located in Koloudeh, Amole, and then they were transferred to the laboratory. The samples were washed with tap water and a few drops of dish washing liquid, 15 minutes and then were washed three times with distilled water. Factorial experiment was performed in a completely randomized design with four replicates (each replicate contained 25 seeds). The factors were: factor A, in culture media containing sterile sand and peat (placed at 105 c ° for 24 hours); factor B, regulating substances including hormone gibberellic acid and potassium nitrate salts; factor C, concentration contains three levels for each materials (gibberellic acid in 3 levels 200, 300 and 400 ppm and potassium nitrate in levels of 0.1, 0.2 and 0.3 mol/l); and factor D contains the suspension time of seeds in 24 and 48 hours, respectively. After a certain time periods, the seeds were out of the solution and they were planted separately for each treatment in plastic pots with a depth of one centimeter. The seeds were watered daily in the morning. Germination were recorded daily during 21 days, and different characteristics of germination vegetation were calculated that they included germination percent, energy of germination, the maximum average of germination during germination period, speed of germination, the average time of germination, germination value and the average of daily germination for germination traits and leaf length, seedling length, root length and stem length with precision of 1 mm, stem dry weight, root dry weight, stem fresh weight, root fresh weight with precision of 0/001 g and the number of leaves for vegetation traits. For calculating the dry weight, the samples were putted in 103 ° C for 24 hours. The analysis of data was carried out with Excel 2007, SPSS 16.0 and the following formula was used.

Table 1: The analysis variance results of germination properties

		GP	GE	PV	GS	MTG	GV	MDG
Bed	f	0.31	0.39	0.23	0.62	0.22	0.92	0.38
	sig	0.57 <sup>ns</sup>	0.53 <sup>ns</sup>	0.62 <sup>ns</sup>	0.43 <sup>ns</sup>	0.64 <sup>ns</sup>	0.33 <sup>ns</sup>	0.53 <sup>ns</sup>
Hormone	f	10.04	1.98	11.25	11.51	6.56	8.76	10.21
	sig	0**	0.12 <sup>ns</sup>	0**	0**	0**	0**	0**
Concentration	f	4.28	3.32	5.05	2.31	2.58	3.07	3.93
	sig	0.01**	0.04*	0**	0.10 <sup>ns</sup>	0.08 <sup>ns</sup>	0.05 <sup>ns</sup>	0.02*
Time	f	9.22	7.05	10.87	12.88	26.78	8.47	9.36
	sig	0**	0**	0**	0**	0**	0**	0**
B.H	f	6.58	1.69	7.82	7.78	4.31	6.63	6.71
	sig	0**	0.14 <sup>ns</sup>	0**	0**	0**	0**	0**
B.C	f	1.46	0.59	3.77	1.56	0.22	2.88	1.61
	sig	0.23 <sup>ns</sup>	0.55 <sup>ns</sup>	0.02*	0.21 <sup>ns</sup>	0.8 <sup>ns</sup>	0.06 <sup>ns</sup>	0.20 <sup>ns</sup>
B.T	f	6.02	7.81	7.06	7.89	22.22	6.17	6.17
	sig	0**	0**	0**	0**	0**	0**	0**
H.C	f	6.57	1.34	6.62	6.64	3.35	5.25	6.43
	sig	0**	0.24 <sup>ns</sup>	0**	0**	0**	0**	0**
H.T	f	6.79	4.56	7.13	9.84	17.05	5.66	6.87
	sig	0**	0**	0**	0**	0**	0**	0**
C.T	f	0.18	0	0.37	0.09	1	0.24	0.14
	sig	0.83 <sup>ns</sup>	0.99 <sup>ns</sup>	0.69 <sup>ns</sup>	0.90 <sup>ns</sup>	0.37 <sup>ns</sup>	0.78 <sup>ns</sup>	0.86 <sup>ns</sup>
B.H.C	f	5.38	1	6.20	6.46	2.63	5.18	5.32
	sig	0**	0.45 <sup>ns</sup>	0**	0**	0**	0**	0**
B.H.T	f	4.41	5.5	4.95	6.18	13.55	4.62	4.52
	sig	0**	0**	0**	0**	0**	0**	0**
H.C.T	f	4.29	3.77	4.12	5.11	7.97	3.34	4.25
	sig	0**	0**	0**	0**	0**	0**	0**
B.H.C.T	f	3.65	4.13	4.12	4.88	9.46	3.59	3.68
	sig	0**	0**	0**	0**	0**	0**	0**

\*\* Significant at the 99% Probability level

\* Significant at the 95% Probability level

ns no significant

GP: germination percent, GE: germination energy, PV: Maximum mean daily germination, GS: germination speed, MTG: Mean time to germination, GV: germination value, MDG: Mean daily germination.

Germination percent=  $n/N \times 100$  {n, Total number of seed germination- N, Total number of seed sown }

Germination energy=  $Mng/N \times 100$  {Mng, maximum of cumulative seed germination percent }

Maximum mean daily germination (PV)=  $cgp/ti$  {cpg, cumulative germination percent- ti, days science sowing }

Germination speed=  $\sum(ni/ti)$  {number of newly germinated seed at time t- ti, number of days since sowing }

Mean time to germination=  $\sum(ni \times ti) / \sum n$

Germination value= final MDG  $\times$  PV

Mean daily germination (MDG)=  $\sum Cpsgt/T$  {cumulative percent of seed germinated at the end of test }

Table 2: The analysis variance results of vegetative properties

		L.L	T.L	R.L	S.L	L.N	S.D.W	R.D.W	S.F.W	R.F.W
Bed	f	55.01	97.82	468.23	34.72	0.58	4.95	0.73	43.62	5.63
	sig	0**	0**	0**	0**	0.45 <sup>ns</sup>	0.02*	0.39 <sup>ns</sup>	0**	0.02*
Hormone	f	0.36	0.42	1.49	2.25	0.47	1.64	0.71	0.35	0.26
	sig	0.78 <sup>ns</sup>	0.74 <sup>ns</sup>	0.22 <sup>ns</sup>	0.08 <sup>ns</sup>	0.22 <sup>ns</sup>	0.18 <sup>ns</sup>	0.54 <sup>ns</sup>	0.79 <sup>ns</sup>	0.85 <sup>ns</sup>
Concentration	f	0.59	0.27	8.54	2.8	3.09	1.98	3.51	1.47	5.81
	sig	0.55 <sup>ns</sup>	0.76 <sup>ns</sup>	0**	0.06 <sup>ns</sup>	0.05*	0.14 <sup>ns</sup>	0.03*	0.23 <sup>ns</sup>	0**
Time	f	0.42	0.68	2.11	1.55	0.14	0.01	0.12	1.07	6.33
	sig	0.74 <sup>ns</sup>	0.57 <sup>ns</sup>	0.10 <sup>ns</sup>	0.21 <sup>ns</sup>	0.94 <sup>ns</sup>	0.39 <sup>ns</sup>	0.89 <sup>ns</sup>	0.36 <sup>ns</sup>	0**
B.H	f	11.67	19.51	95.26	9.15	0.42	2.60	0.97	10.34	2.15
	sig	0**	0**	0**	0**	0.18 <sup>ns</sup>	0.03*	0.43 <sup>ns</sup>	0**	0.06 <sup>ns</sup>
B.C	f	1.25	1.14	2.65	0.10	2.08	0.15	1.04	0.16	1.99
	sig	0.28 <sup>ns</sup>	0.32 <sup>ns</sup>	0.07 <sup>ns</sup>	0.89 <sup>ns</sup>	0.13 <sup>ns</sup>	0.85 <sup>ns</sup>	0.35 <sup>ns</sup>	0.85 <sup>ns</sup>	0.14 <sup>ns</sup>
B.T	f	13.21	20.03	108.94	7.71	0.16	3.15	4.35	11.46	5.45
	sig	0**	0**	0**	0**	0.97	0**	0**	0**	0**
H.C	f	0.34	0.36	33.12	1.47	4.12	1.71	1.54	0.48	1.27
	sig	0.93 <sup>ns</sup>	0.91 <sup>ns</sup>	0**	0.18 <sup>ns</sup>	0**	0.11 <sup>ns</sup>	0.16 <sup>ns</sup>	0.84 <sup>ns</sup>	0.27 <sup>ns</sup>
H.T	f	0.31	0.56	41.46	1.99	0.22	2.77	7.23	1.67	5.20
	sig	0.90 <sup>ns</sup>	0.72 <sup>ns</sup>	0**	0.08 <sup>ns</sup>	0.83 <sup>ns</sup>	0**	0**	0.14 <sup>ns</sup>	0**
C.T	f	0.74	1.55	0.11	4.95	4.42	1.41	1.61	2.96	0.83
	sig	0.47 <sup>ns</sup>	0.21 <sup>ns</sup>	0.89 <sup>ns</sup>	0**	0.01**	0.24 <sup>ns</sup>	0.20 <sup>ns</sup>	0.05 <sup>ns</sup>	0.43 <sup>ns</sup>
B.H.C	f	4.79	7.60	45.11	4.51	2.82	1.63	1.57	4.12	2.54
	sig	0**	0**	0**	0**	0**	0.90 <sup>ns</sup>	0.10 <sup>ns</sup>	0**	0**
B.H.T	f	7.35	11.12	62.80	5.60	1.84	2.32	4.41	8.31	4.59
	sig	0**	0**	0**	0**	0.07 <sup>ns</sup>	0.02*	0**	0**	0**
H.C.T	f	0.29	0.55	0.86	2.04	4.70	1.53	4.26	11.48	3.26
	sig	0.99 <sup>ns</sup>	0.88 <sup>ns</sup>	0.58 <sup>ns</sup>	0.02*	0**	0.12 <sup>ns</sup>	0**	0**	0**
B.H.C.T	f	3.51	5.41	32.18	4.73	3.02	4.47	2.95	4.48	3.53
	sig	0**	0**	0**	0**	0**	0.02*	0**	0**	0**

L.L: leaf length, S.L: seedling length, R.L: root length, S.L: stem length (cm), L.N; leaf number, S.D.W: stem dry weight, R.D.W: root dry weight, S.F.W; stem fresh weight, R.F.W: root fresh weight

## Results

The analysis of variance results of germination properties have been presented in Table (1) and the results of comparing their average have been presented in Table (3). As it has been observed, the most of these properties are affected by the most of factors in 99% significance level. Soaking of seeds in potassium nitrate with a concentration of 0.1% for 48 hours and culturing in Peat made the best results (for example 99% of germination toward 58% in control). Also, it has been obtained good results, spatially, in percent of germination (92%) by treating with gibberellic acid in concentration of 300 ppm for 48 hours and culturing in this

bed. But, it seems due to the lower relative increase in values of other attributes compared to potassium nitrate, potassium nitrate was the most suitable ameliorative with a concentration of 0.1% and time of soaking for 48 in Peat bed.

The analysis variance results of vegetative properties have been presented in Table (2) and the results of comparing their average have been presented in Table (4). It is possible not to introduce a specific treatment for vegetative properties as the best treatment, because these properties showed so different values in variant treatments such that in some case the treated seeds and in other cases the control seeds had high value. For example the length of root was higher in treatment by potassium nitrate with a concentration of 0.2 for 24 hours in Peat, while it was less than the control seed in sand. The condition was completely contrary for the length of stem and it was higher in mentioned treatment in sand, while it had lower value than the control seeds in Peat. The wet weight of root was higher in Peat bed in treatment by gibberellic acid with a concentration of 300 and 400 ppm for 48 hours and it was lower than the control seeds within 24 hours in the same treatment.

Table 3: comparing average of germination properties

Bed	regulating substances	Conce	Time	MDG	GV	MTG	GS	PV	GE	GP		
Sand	GA <sub>3</sub>	200	24	0.92 <sup>bcdefg</sup>	3.73 <sup>bcdef</sup>	16.40 <sup>ghijk</sup>	1.20 <sup>abcdefg</sup>	4.00 <sup>bcdef</sup>	45 <sup>cdef</sup>	78 <sup>abcdef</sup>		
		200	48	0.96 <sup>abcdef</sup>	3.98 <sup>bcde</sup>	16.31 <sup>ghijk</sup>	1.26 <sup>abcdef</sup>	4.11 <sup>bcde</sup>	49 <sup>cde</sup>	81 <sup>abcde</sup>		
		300	24	0.89 <sup>bcdefg</sup>	3.42 <sup>cdef</sup>	16.38 <sup>ghijk</sup>	1.15 <sup>cdefgh</sup>	3.79 <sup>bcdef</sup>	37 <sup>ef</sup>	75 <sup>bcdef</sup>		
		300	48	0.98 <sup>abcd</sup>	4.08 <sup>bcde</sup>	17.00 <sup>cdefg</sup>	1.25 <sup>abcdef</sup>	4.11 <sup>bcde</sup>	66 <sup>abcd</sup>	83 <sup>abcd</sup>		
		400	24	1.03 <sup>ab</sup>	4.63 <sup>abc</sup>	16.51 <sup>fghijk</sup>	1.34 <sup>abcd</sup>	4.46 <sup>ab</sup>	57 <sup>bcde</sup>	87 <sup>ab</sup>		
		400	48	1.02 <sup>abc</sup>	4.55 <sup>abc</sup>	16.46 <sup>ghijk</sup>	1.33 <sup>abcd</sup>	4.40 <sup>abc</sup>	63 <sup>abcd</sup>	86 <sup>ab</sup>		
	KNO <sub>3</sub>	0.1	24	0.91 <sup>bcdefg</sup>	3.58 <sup>cdef</sup>	16.07 <sup>hijkl</sup>	1.20 <sup>abcdefg</sup>	3.90 <sup>bcdef</sup>	48 <sup>cdef</sup>	77 <sup>bcdef</sup>		
		0.1	48	0.82 <sup>efgh</sup>	2.87 <sup>efg</sup>	17.55 <sup>bcd</sup>	1.00 <sup>ghi</sup>	3.50 <sup>ef</sup>	47 <sup>cdef</sup>	69 <sup>defg</sup>		
		0.2	24	0.94 <sup>abcdefg</sup>	3.76 <sup>bcdef</sup>	16.36 <sup>ghijk</sup>	1.23 <sup>abcdef</sup>	3.94 <sup>bcdef</sup>	49 <sup>cde</sup>	79 <sup>abcdef</sup>		
		0.2	48	0.84 <sup>defg</sup>	3.16 <sup>defg</sup>	16.42 <sup>ghijk</sup>	1.09 <sup>efgh</sup>	3.74 <sup>cdef</sup>	33 <sup>ef</sup>	71 <sup>cdef</sup>		
		0.3	24	1.02 <sup>abc</sup>	4.47 <sup>abc</sup>	16.75 <sup>efghij</sup>	1.30 <sup>abcde</sup>	4.35 <sup>abc</sup>	57 <sup>bcde</sup>	86 <sup>ab</sup>		
		0.3	48	0.92 <sup>bcdefg</sup>	3.60 <sup>cdef</sup>	17.32 <sup>cde</sup>	1.13 <sup>defgh</sup>	3.84 <sup>bcdef</sup>	46 <sup>cdef</sup>	78 <sup>abcdef</sup>		
		Pit	GA <sub>3</sub>	200	24	0.93 <sup>abcdefg</sup>	3.76 <sup>bcdef</sup>	16.90 <sup>defgh</sup>	1.18 <sup>abcdefg</sup>	4.00 <sup>bcdef</sup>	62 <sup>abcd</sup>	78 <sup>abcdef</sup>
				200	48	0.86 <sup>cdefg</sup>	3.38 <sup>cdef</sup>	17.27 <sup>cdef</sup>	1.07 <sup>fghi</sup>	3.84 <sup>bcdef</sup>	53 <sup>bcde</sup>	73 <sup>bcdef</sup>
300	24			0.97 <sup>abcdef</sup>	3.86 <sup>bcdef</sup>	15.92 <sup>jkl</sup>	1.32 <sup>abcd</sup>	3.95 <sup>bcdef</sup>	34 <sup>ef</sup>	84 <sup>abcd</sup>		
300	48			1.10 <sup>a</sup>	4.90 <sup>ab</sup>	16.86 <sup>defghi</sup>	1.40 <sup>a</sup>	4.45 <sup>ab</sup>	69 <sup>abc</sup>	92 <sup>a</sup>		
400	24			0.93 <sup>abcdefg</sup>	3.81 <sup>bcdef</sup>	15.73 <sup>kl</sup>	1.27 <sup>abcdef</sup>	4.05 <sup>bcde</sup>	36 <sup>ef</sup>	79 <sup>abcdef</sup>		
400	48			1.00 <sup>abcd</sup>	4.16 <sup>bcd</sup>	18.12 <sup>ab</sup>	1.17 <sup>bcdefg</sup>	4.10 <sup>bcde</sup>	74 <sup>ab</sup>	84 <sup>abc</sup>		
KNO <sub>3</sub>	0.1		24	0.98 <sup>abcde</sup>	4.24 <sup>bcd</sup>	15.33 <sup>l</sup>	1.38 <sup>ab</sup>	4.28 <sup>bc</sup>	25 <sup>f</sup>	83 <sup>abcd</sup>		
	0.1		48	1.09 <sup>a</sup>	5.47 <sup>a</sup>	16.91 <sup>defg</sup>	1.37 <sup>abc</sup>	4.97 <sup>a</sup>	84 <sup>a</sup>	92 <sup>a</sup>		
	0.2		24	0.91 <sup>bcdefg</sup>	3.54 <sup>cdef</sup>	15.96 <sup>jkl</sup>	1.23 <sup>abcdef</sup>	3.82 <sup>bcdef</sup>	44 <sup>def</sup>	77 <sup>bcdef</sup>		
	0.2		48	0.81 <sup>fgh</sup>	3.43 <sup>cdef</sup>	17.73 <sup>abc</sup>	0.97 <sup>hi</sup>	3.53 <sup>def</sup>	56 <sup>bcde</sup>	68 <sup>efg</sup>		
	0.3		24	0.97 <sup>abcdef</sup>	4.15 <sup>bcde</sup>	16.06 <sup>ijkl</sup>	1.30 <sup>abcde</sup>	4.22 <sup>bcd</sup>	51 <sup>bcde</sup>	82 <sup>abcd</sup>		
	0.3		48	1.00 <sup>abcd</sup>	4.11 <sup>bcde</sup>	17.75 <sup>abc</sup>	1.21 <sup>abcdefg</sup>	4.1 <sup>bcde</sup>	69 <sup>abc</sup>	84 <sup>abc</sup>		
	sand control				0.80 <sup>gh</sup>	2.69 <sup>fg</sup>	18.44 <sup>a</sup>	0.91 <sup>i</sup>	3.35 <sup>fg</sup>	37 <sup>ef</sup>	67 <sup>fg</sup>	
	Pit control				0.70 <sup>h</sup>	2.08 <sup>g</sup>	16.37 <sup>ghijk</sup>	0.90 <sup>i</sup>	2.85 <sup>g</sup>	37 <sup>ef</sup>	58 <sup>g</sup>	

### Conclusion

Germination and early growth is one of the most important phenological stages of plant growth and determinative of success rate in plant systems (Forcella et al, 2000). Important factors affecting this stage are the power (Eisvand & Alizadeh, 2003), speed and simultaneity of germination and early seedling growth of seeds that enhance seed efficacy by increasing the percentage of seedlings with acceptable growth parameters. If seeds cannot germinate in the long term, there is a possibility to lose them due to function of organisms and microorganisms. It is apparent that germination percent should be associated with the time factor, which based on this fact, the germination rate is used (Mexal, 1987). The morphological characteristics of the plants are another important factor in success. The length of plantlet is the criteria of vigor and in many species, the correlation between length and specified seedling vigor were used as criteria for assessing the growth and plantlet vigor (Hampton & Tekrony, 1995).

It is necessary to note, that the initial height can result in the release of plantlet from ground shade which can rescue quickly escape from the shadow of the forest cover (Khosravi, 1996). Previous studies suggest positive effects of nitrate compounds and gibberellins on growth characteristics and seed germination of different species of pine (Ghildiyal et al, 2009; Little & MacDonald, 2003; Larionova, 1997; Barzdajn, 1986). These compounds with affecting on the levels of other hormones influence on seed physiological processes and stimulate seed germination (Thomas & Sambrooks., 1985).

In this study, the effects of combination of gibberellic acid and potassium nitrate, concentration, time of suspension and planting bed of pine storage seeds have been investigated. The results showed that storage seeds like new seeds were affected by the concentrations of these substances in different times. Seed germination traits were improved in all treatments, which corresponded with the results of the mentioned studies. But the growth characteristics in different treatments showed different results, so that in some cases, treated seeds had higher values and in other cases these characteristics were higher in control seeds. Many studies have been done on the action of these substances. Some researchers believe that GA enhances RNA polymerase, and thus increase the rate of transcription of some portions of DNA and stimulates the production of enzymes which are required in germination. These enzymes cause changes in production of some proteins and finally, they stimulate the production of hydrolysis enzymes of seed storage molecules, such as  $\alpha$ -amylase.

Table 4: comparing average of vegetative properties

Bed	regulating substances	Con ce	Tim e	L.L	T.L	R.L	S.L	L.N	S.D.W	R.D.W	S.W.W	R.W.W		
Sand	GA <sub>3</sub>	200	24	2.50 <sup>a</sup>	10 <sup>efgh</sup>	3.73 <sup>h</sup>	3.76 <sup>abc</sup>	6.20 <sup>cdef</sup>	0.16 <sup>abc</sup>	0.05 <sup>de</sup>	1.52 <sup>bcdef</sup>	0.50 <sup>bcd</sup>		
		200	48	2.42 <sup>abcdef</sup>	9.73 <sup>gh</sup>	4.02 <sup>fgh</sup>	3.30 <sup>cdefg</sup>	6.65 <sup>abc</sup>	0.14 <sup>abc</sup>	0.05 <sup>de</sup>	1.56 <sup>abcdef</sup>	0.39 <sup>cdef</sup>		
		300	24	2.58 <sup>abcd</sup>	11.20 <sup>cdefgh</sup>	5.10 <sup>f</sup>	3.51 <sup>abcdef</sup>	6.71 <sup>abc</sup>	0.18 <sup>ab</sup>	0.07 <sup>bcde</sup>	1.66 <sup>abcde</sup>	0.43 <sup>cdef</sup>		
		300	48	2.77 <sup>a</sup>	10.90 <sup>cdefgh</sup>	4.39 <sup>fgh</sup>	3.56 <sup>abcde</sup>	6.71 <sup>abc</sup>	0.19 <sup>ab</sup>	<b>0.06</b> <sup>cde</sup>	1.68 <sup>abcde</sup>	0.41 <sup>cdef</sup>		
	KNO <sub>3</sub>	400	24	2.31 <sup>abcdefgh</sup>	9.80 <sup>fgh</sup>	3.80 <sup>gh</sup>	3.67 <sup>abcd</sup>	6.32 <sup>bcd</sup>	0.19 <sup>ab</sup>	0.06 <sup>bcd</sup>	1.60 <sup>abcde</sup>	0.47 <sup>cde</sup>		
		400	48	2.60 <sup>abcd</sup>	10.20 <sup>efgh</sup>	3.87 <sup>gh</sup>	3.72 <sup>abcd</sup>	6.05 <sup>def</sup>	0.21 <sup>a</sup>	0.06 <sup>cde</sup>	1.74 <sup>abcd</sup>	0.48 <sup>cd</sup>		
		0.1	24	2.73 <sup>ab</sup>	11.33 <sup>cdefgh</sup>	4.91 <sup>fg</sup>	3.70 <sup>abcd</sup>	6.90 <sup>ab</sup>	0.20 <sup>ab</sup>	0.06 <sup>bcd</sup>	1.89 <sup>ab</sup>	0.40 <sup>cdef</sup>		
		0.1	48	2.30 <sup>abcdefgh</sup>	8.86 <sup>h</sup>	3.68 <sup>h</sup>	2.87 <sup>fgh</sup>	6.74 <sup>abc</sup>	0.17 <sup>ab</sup>	0.06 <sup>cde</sup>	1.30 <sup>cdefgh</sup>	0.40 <sup>cdef</sup>		
		0.2	24	2.48 <sup>abcde</sup>	11.01 <sup>cdefgh</sup>	4.40 <sup>fgh</sup>	4.13 <sup>a</sup>	6.49 <sup>abcd</sup>	0.18 <sup>ab</sup>	0.09 <sup>ab</sup>	1.78 <sup>abc</sup>	0.68 <sup>ab</sup>		
		0.2	48	2.63 <sup>abc</sup>	10.10 <sup>efgh</sup>	4.08 <sup>fgh</sup>	3.37 <sup>bcd</sup>	6.62 <sup>abcd</sup>	0.18 <sup>ab</sup>	0.05 <sup>de</sup>	1.48 <sup>bcdef</sup>	0.42 <sup>cdef</sup>		
		0.3	24	2.47 <sup>abcde</sup>	10.68 <sup>defgh</sup>	5.14 <sup>f</sup>	3.06 <sup>defg</sup>	6.75 <sup>abc</sup>	0.16 <sup>abc</sup>	0.10 <sup>ab</sup>	1.50 <sup>bcdef</sup>	0.78 <sup>a</sup>		
		0.3	48	2.80 <sup>a</sup>	11.03 <sup>cdefgh</sup>	4.27 <sup>fgh</sup>	3.95 <sup>ab</sup>	6.76 <sup>abc</sup>	0.20 <sup>a</sup>	0.05 <sup>de</sup>	2.03 <sup>a</sup>	0.54 <sup>bcd</sup>		
		Pit	GA <sub>3</sub>	200	24	2.16 <sup>cdefgh</sup>	12.20 <sup>bcd</sup>	7.20 <sup>de</sup>	2.76 <sup>gh</sup>	5.70 <sup>f</sup>	0.17 <sup>ab</sup>	0.06 <sup>cde</sup>	1.21 <sup>efgh</sup>	0.41 <sup>cdef</sup>
				200	48	2.02 <sup>efgh</sup>	15.60 <sup>a</sup>	7.68 <sup>bcd</sup>	3.08 <sup>defg</sup>	6.54 <sup>abcd</sup>	0.14 <sup>abc</sup>	0.05 <sup>de</sup>	1.05 <sup>fgh</sup>	0.35 <sup>def</sup>
300	24			2.24 <sup>bcd</sup>	14.42 <sup>ab</sup>	8.65 <sup>ab</sup>	3.52 <sup>abc</sup>	6.66 <sup>abc</sup>	0.18 <sup>ab</sup>	0.08 <sup>abcd</sup>	1.27 <sup>cdefgh</sup>	0.48 <sup>cd</sup>		
300	48			1.88 <sup>gh</sup>	13.20 <sup>abcd</sup>	7.83 <sup>bcd</sup>	3.37 <sup>bcd</sup>	6.51 <sup>abcd</sup>	0.21 <sup>a</sup>	0.07 <sup>bcd</sup>	1.49 <sup>bcdef</sup>	0.42 <sup>cdef</sup>		
KNO <sub>3</sub>	400		24	2.12 <sup>cdefgh</sup>	14.21 <sup>ab</sup>	8.59 <sup>abc</sup>	3.30 <sup>cdefg</sup>	6.67 <sup>abc</sup>	0.18 <sup>ab</sup>	0.07 <sup>abcd</sup>	1.36 <sup>cdefgh</sup>	0.53 <sup>bcd</sup>		
	400		48	1.83 <sup>h</sup>	12.33 <sup>bcd</sup>	7.40 <sup>cde</sup>	3.15 <sup>cdefg</sup>	5.87 <sup>ef</sup>	0.18 <sup>ab</sup>	0.07 <sup>bcd</sup>	1.20 <sup>efgh</sup>	0.42 <sup>cdef</sup>		
	0.1		24	2.10 <sup>defgh</sup>	13.32 <sup>abc</sup>	8.33 <sup>abcd</sup>	2.92 <sup>fgh</sup>	6.69 <sup>abc</sup>	0.16 <sup>abc</sup>	0.08 <sup>abc</sup>	1.23 <sup>defgh</sup>	0.53 <sup>bcd</sup>		
	0.1		48	2.30 <sup>abcdefgh</sup>	13.99 <sup>ab</sup>	6.47 <sup>e</sup>	2.90 <sup>fgh</sup>	6.47 <sup>abcd</sup>	0.12 <sup>bc</sup>	0.06 <sup>cde</sup>	0.89 <sup>hi</sup>	0.36 <sup>def</sup>		
	0.2		24	2.31 <sup>abcdefgh</sup>	13.86 <sup>ab</sup>	8.63 <sup>ab</sup>	3.18 <sup>cdefg</sup>	6.70 <sup>abc</sup>	0.19 <sup>ab</sup>	0.06 <sup>cde</sup>	1.50 <sup>bcdef</sup>	0.40 <sup>cdef</sup>		
	0.2		48	1.86 <sup>gh</sup>	12.44 <sup>bcd</sup>	8.21 <sup>bcd</sup>	2.42 <sup>h</sup>	7.06 <sup>a</sup>	0.10 <sup>c</sup>	0.04 <sup>e</sup>	0.55 <sup>i</sup>	0.25 <sup>f</sup>		
	0.3		24	2.05 <sup>efgh</sup>	14.12 <sup>ab</sup>	9.23 <sup>a</sup>	2.83 <sup>gh</sup>	6.75 <sup>abc</sup>	0.16 <sup>abc</sup>	0.10 <sup>a</sup>	1.23 <sup>defgh</sup>	0.60 <sup>bc</sup>		
	0.3		48	1.94 <sup>fgh</sup>	13.09 <sup>abcd</sup>	8.36 <sup>abcd</sup>	2.78 <sup>gh</sup>	6.60 <sup>abcd</sup>	0.14 <sup>abc</sup>	0.05 <sup>de</sup>	0.96 <sup>ghi</sup>	0.27 <sup>ef</sup>		
	sand control				2.48 <sup>abcde</sup>	11.03 <sup>cdefg</sup>	4.85 <sup>fgh</sup>	3.70 <sup>abcd</sup>	6.60 <sup>abcd</sup>	0.20 <sup>ab</sup>	0.05 <sup>de</sup>	1.41 <sup>bcdefg</sup>	0.46 <sup>cde</sup>	
	Pit control				2.34 <sup>abcdefg</sup>	12.78 <sup>bcd</sup>	7.72 <sup>ab</sup>	3.21 <sup>cdefg</sup>	6.42 <sup>bcd</sup>	0.15 <sup>abc</sup>	0.06 <sup>cde</sup>	1.33 <sup>cdefgh</sup>	0.44 <sup>cdef</sup>	

These enzymes catalyze the reactions necessary for producing energy and structural components necessary for growth and embryo presence (Harberd & Peng, 2002). Also, they alter the flow of some ions such as K<sup>+</sup> and Ca<sup>2+</sup> from the membrane and these changes cause to transfer special signals and stimulate synthesis or activity of metabolites and germination stimulator enzymes (Thomas & Sambrooks., 1985) and thus it induces germination. Through increasing activity of Catechol oxidase in stimulating germination (Chiwocha et al, 2005) and neutralizing inhibitory effect of Abscisic Acid, as well as increasing value of germination, gibberellin can improve the rate of germination (Kabar, 1998), repair the lesions of seeds, and finally, improve the homogeneity of germination and appearance of seedlings (omidi et al, 2005). Thus, it seems by increasing time of gibberellin up taking and its level in seed, balance between inhibitors and stimulators goes into increase stimulators and then viability and germination of seed increase. This is in agreement with the results of our study. About potassium nitrate, it should be notice that the results of other studies indicate increasing sensitivity to light by organic nitrates (Toole & Toole, 1955). Some of scientists believe to exist a relationship between effect of nitrates or nitric oxide (NO) and activity of phytochromes in germination (Giba et al., 1998). Potassium nitrate appears its stimulatory effect on germination when there is phytochrome A in seeds actively. The possible explanation for effect of nitrates and nitric oxide in germination related to phytochrome is direct relationship with phytochrome Apo protein. NO<sub>3</sub> changes the physiochemical characterizations of phytochrome by binding to it and stimulates germination (Hilhorst, 1990). Furthermore, it is possible nitrogen compounds facilitate the intracellular interaction or they are intermediates for this interactions. Bed was another experiment factor that had a significant effect on only a few growing characterizations. Length of root and total length of seedling in Peat bed were higher, but other growing parameters were higher in sand bed. Soft and porous tissue of Peat provides the better condition for elongation of roots, but in sand, with a power to hold more water, there is more time for seedlings and need long roots reach more water becomes less, thus plant energy is spent on stem elongation. Then roots in sand bed are thicker and have more weight. But roots in Peat bed are so thinner and lighter. Combination of the four assessment factors had a significant effect on all features including vegetative and seed germination. The best results obtained when the treatment had 1% potassium nitrate and gibberellic acid in concentration of 300 ppm for 48 hours in Peat bed to improve germination. Also, treatment with 0.3 % potassium nitrate for 24 hours in Peat was best to improve vegetative factors (the number of leaves, total length, length of root and fresh and dry weight of root). The best results, for other characterizations, were made in the same treatment for 48 hours in sand bed. Generally, gibberellic acid treatment with conditions listed can be used as a common treatment for improving all of parameters. Afterwards, also, treatment with 0.3% potassium nitrate, with both times in sand, makes acceptable results. So, as regard to the different price between the gibberellic acid and potassium nitrate, use of potassium nitrate is economical.

## Reference

- Basra, S.M.A., Ahmad, N., Khan, M.M., Iqbal, N., Cheema, M.A. 2003. Assessment of cottonseed deterioration during accelerated ageing. Seed Sci. Technol. 31, 531-540.
- Barzdajn, W. Effect of aqueous solutions of NaCl, KNO<sub>3</sub> and urea on the germination of *Scots pine* seed, Sylwan, Vol. 130, No. 9, (1986) pp. 39-45.

- Biswas, P.K., Bonamy, P.A. and Paul, K.b. 1972. Germination promotion of Loblolly Pine and Baldcypress seeds by stratification and chemical treatments. *Physiologia Plantarum* 1972 Vol. 27 No. 1 pp. 71-76
- Chiwocha, S.D.S., Culter, A.J., Abrams, S.R., Ambrose, S.J., Yang, J., Ross, A.R.S., Kermode, A.R. 2005. The *ertl-2* mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist chilling and germination. *Plant Journal*. 42: 35-45.
- Coin, L., M. Vaissiere, A. Noirot, A. Charrier, and S. Hamon. 1995. Comparative effects of natural and accelerated ageing on barley seeds (*Hordeum vulgare* L.). *Seed Sci. Technol.* 23:673-688.
- Eisvand, H., and Alizadeh, M.A., 2003. Evaluation of some physiological parameters of seed quality (germination percentage, germination rate index cotton) *Dracocephalum moldavica* L. Under test conditions, premature aging. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research*. 19 (2): 249-255. (In Persian)
- Finkelstein, R., Reeves, W., Arizumi, T. & Steber, C. (2008). Molecular aspects of seed dormancy. *Annual Review of Plant Biology*, 59, 387-415.
- Forcella, F., Benesch Arnold, R.L., Sanchez, R. Ghersa, C.M. 2000. Modeling seedling emergence. *Field. Crop. Res.* 67: 123-139.
- Ghildiyal, S. K., Sharma, C. M., Khanduri, V. P. 2009. Effect of pre-soaking and pre-chilling treatments on seed germination of *Pinus roxburghii* provenances from western Himalaya, India. *Journal of Forestry Research*. Vol. 20 No. 4 pp. 323-330, ii.
- Giba, Z., Grubisic, D., Todorovic, S., Stojakovic, D. and Konjevic R. 1998. Effects of nitric oxide-releasing compounds on phytochrome-controlled germination of Empress tree seeds. *Plant growth regulation*, 26: 175-181.
- Golzar, S, A. M. Khan and I. A. Ungar. 2001. Effect of salinity and temperature on the germination of *urochondra setulosa*. *Seed sci. and Technol.* 29, 21-29.
- Hampton, J. G. and D. M. Tekrony. 1995. *Handbook of Vigour Test Methods* (3rd.Ed.). International Seed Testing Association (ISTA).Zurich, Swirztland.
- Harberd, N.P., and Peng, J. 2002. The role of GA-mediated signaling in the control of germination. *Science*. 5: 376-381.
- Hilhorst, H.W.M. 1990. Seed dormancy and germination: light and nitrate. PhD thesis, University of Wageningen, The Netherlands.
- Justice, O.L., and L.N. Bass. 1979. Principles and practices of seed storage. U.S. Department of Agriculture. 289 p.
- Kabar, K., 1998. Comparative effects of kinetin, benzyladenine and gibberellic acid on abscisic acid inhibited seed germination and seedling growth of red pine and arbor vitae. *Turk. J. Bot.* 22:1-6.
- Kermode, A. R. (2005). Role of abscisic acid in seed dormancy. *Journal of Plant Growth Regulation*, 24, 319-344.
- Khosravi, M. 1996. *Seed Ecology*. Publication of Mashhad University. 182 pp. (In Persian)
- Larionova, N. A. *Lesnoe Khozyaistvo* 1997 No. 6 pp. 28-30
- Lavana, S. K.; Singh, R. P. 2006. Influence of Cone Collection Date on seed and germination Characteristics in Blue Pine, *Pinus wallichiana* Ab.jacks. *Virendra Singh. Indian Forester* 2006 Vol. 132 No. 8 pp. 1024-1028.
- Little, C. H. A.; MacDonald, J. E. *Tree Physiology* 2003 Vol. 23 No. 2 pp. 73-83.
- Lotfi, M., Aliabadi, A., Rezvani, A., and Amiri, R., 2011. Effect of seed priming with different materials and osmotic potentials on germination of melon. *Journal of Crops Improvement*. 13 (1): 65-74. (In Persian)
- Mexal J.G. and Fisher J.T. 1987. Size hierarchy in conifer seedbeds. I. Time of emergence. *New Forests*1: 187-196.
- Nasirzadeh, A.R., Jokar, L., and Negahdari, H., 2007. Effects of gibberellic acid on growth of oak (*Quercus brantii* var. *persica*) seedlings. *Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research*. 15 (3): 253-264. (In Persian)
- Omidi H., A., Sorushzadeh, A. Salehi and F. Ghezeli. 2005. Evaluation of Priming pretreatments on germination rapeseed. *Agricultural Science and Technology*, 19(2): 1-10.
- Parera, CA. and Cantliff. DJ., (1994) Pre-sowing seed priming. *In: J Janick (ed.). Horticultural Reviews* 16: 119-141.
- Qiang, w., Xiao, R. and Qichuan, Y. 2005. Study on the effect of plant hormones and prechilled treatment to break dormancy and germination of *Rhodiola rosea* seeds. *Journal of Zhejiang University Agriculture and Life Sciences* .31: 423- 432.
- Salisbury, F.B. and Ross, C.W., 1978. *Plant Physiology*. Wadsworth Publishing Company, Inc, 422p.
- Sasaki, S., Asakawa, S. 1974. Effect of monochromatic lights on the germination of Pine seeds processed in darkness. *Journal of the Japanese Forestry Society* Vol. 56 No. 10 pp. 361-365.
- Soltani, A. 2004. Chlorophyll fluorescence and its application. Internal press. University of Agricultural Science and natural resource, Gorgan, Iran.
- Soltani, A., E. Zeinali, S. Galeshi, and N. Latifi. 2001. Genetic variation for and interrelationships among seed vigor traits in wheat from the Caspian Sea coast of Iran. *Seed Sci. Technol.* 29:653-662.
- Thomas, T.H. 1990. Hormonal involvement in photoregulation of celery seed dormancy. *Monograph- British Society for Plant Growth Regulation*. 20:51-59.
- Thomas, T.H., Sambrooks, D.F. 1985. Possible control of gibberellin-induced release of temperature dependent primary dormancy in seeds of celery (*Apium graveolens*) by trans membrane ion fluxes. *Plant Growth Regulation*. 3: 191-199.
- Toole, E.H., Toole, V.K., Bortwick, H.A. and Hendricks, S.B. 1955. Photocontrol of *Lepidium* seed germination. *Plant Physiology*, 30: 15-21.