

Full Length Research Paper

# Comparison of two culture media for breaking seed dormancy and germination improvement in four species of *Linum* L.

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The aim of this study was to compare the effects of different treatments in breaking dormancy and to increase germination percentage and days to germination in two media of water with agar and Murashige and Skoog (MS) growth medium in four species of *Linum* L. namely, *L. mucronatum*, *L. nervosum*, *L. album* and *L. austriacum*. Stages of breaking dormancy were incorporated with the exogenous application of gibberellic acid (GA<sub>3</sub>) (400, 800 and 1600 part per million (ppm) for periods of 72, 96, 144 and 168 h at 4°C; sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (25, 40 and 50%) for periods of 10, 15, 20 and 25 min and washing in 70°C running water for 24, 48, 72 and 96 h and some combined treatments such as H<sub>2</sub>SO<sub>4</sub> with GA<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub> with washing in 70°C running water treatments. Germination of four species of *Linum* significantly increased in higher concentrations of GA<sub>3</sub> (1600 ppm). The best result in H<sub>2</sub>SO<sub>4</sub> treatments was recorded at 25% concentration for 25 min. Also, the percentage germination and days to germination in each of the four species were higher and faster in water with agar medium in comparison to MS growth medium. These results suggest that the *Linum* seeds have exogenous and endogenous dormancy.

**Key words:** Dormancy, germination, MS media, *Linum* species, water + agar medium.

## INTRODUCTION

The genus *Linum* is a typical genus of the family Linaceae (DC.) Dumort. The Linaceae family is geographically widespread with about 300 species worldwide (Diederichsen and Richards, 2003). Several of the species are in the form of shrubs and occur in tropical areas, while perennial and annual species are found in temperate areas of the world. The genus *Linum* has two main clusters, one mainly consisting of the sections *Linopsis* and *Syllinum* and the other contains section *Linum* L. *Linum album* Ky. ex Boiss. (Linaceae), known as 'Katan-e-Golsefid' in Persian, is an endemic herbaceous perennial plant widely distributed in mountainous areas, sandy slopes and sandy-clay soils in fields at an altitude of 1200 to 3200 m in Irano-Turanian

regions (Rechinger, 1974; Jalili and Jamzad., 1999). During germination process, part of the embryo, usually the radicle extends to penetrate the structures that surround it and this process is followed by adequate water and oxygen at a suitable temperature. Dormancy is defined as a state of the seed that does not permit germination, although conditions for germination may be provided (temperature, water and oxygen). Thus dormancy effectively delays germination. Conditions required for breaking dormancy and allowing subsequent germination are often very different from those that are necessary for growth or survival of the autotrophic life stage of a plant. Timing of seed germination can be critical for the survival of natural plant populations, and dormancy mechanisms play a major role in this time.

Various methods have been used by seed scientists and technologists to break seed dormancy. Stratification plays an important role as a stimulator that helps to break dormancy (Bewley and Black, 1994; Agrowal and

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Dadlani, 1995; Hartmann et al., 1997). In order to accelerate this method, it can be combined with some treatments such as chemical substances or mechanical seed coat removal (Mehanna et al., 1985; Martinez-Gomez and Dicenta, 2001). Many investigators have studied effects of exogenous growth regulators on seed germination. Gibberellins eliminated the chilling requirements of peach and apple seeds and increased their germination (Rouskas et al., 1980; Mehanna et al., 1985). The beginning of the embryo dormancy is associated with accumulation of growth inhibitors such as abscisic acid (ABA), while the breaking of dormancy with a shift in the balance of growth regulators towards growth promoters such as gibberellic acid (GA<sub>3</sub>) that overcome the effect of growth inhibitors (Rehman and Park, 2000).

Recently, the improvement of scientific and economical value and tissue culture studies of *Linum* species has shown seed germination studies to be important. For this reason, effective factors in seed germination were studied in the present investigation using two media, MS and water + agar.

## MATERIALS AND METHODS

This study was carried out in the Biotechnology and Science Institute of Urmia University. Freshly harvested seeds of four species of *Linum* were collected from west and east Azarbaijan provinces on the 15th of July 2010, and were identified by GeneBank of Agricultural Research Institute and then transported to laboratory. Seeds were tested for germination and viability. The viability percentage estimation was carried out through tetrazolium test, which indicated the presence of more than (95%) viable seed. The seeds were then soaked in half the volume of cold water for 24 h to germination improvement.

### Medium and culture conditions

The basic nutrient medium (BM) consisted of Murashige and Skoog, 1962 (MS) medium including vitamins, macro and micro elements, 3% sucrose and 0.7% agar. The pH was adjusted to 5.7 with KOH (0.1 N) or HCl (1 N) before autoclaving. The other medium included only water and 7% agar. Both media were autoclaved for 20 min at 121°C.

### Treatment with acid scarification

The seeds were scarified with concentrations (25, 40 and 50%) of commercial grade sulfuric acid for 10, 15, 20 and 25 min. After complete washing, the seeds were placed in Petri dish (100 × 15 mm) with water and agar medium and MS growth medium at a rate of 30 seeds per dish. The Petri dishes were wrapped with a strip of Para film around the edge to prevent evaporation. Date and treatment type were recorded on the each Petri dish (Dahlquist, 2004).

The experiment was arranged in a randomized split plot design with four replicates. Germination was carried out in a chamber at 22 ± 2°C with 70% of humidity under dark condition. The seeds were observed every day for germination. Germination was recorded when the white radicle (root) emerged from the seed and its length was 1 cm. The number of germinated seeds in each dish were counted every day and recorded daily on a prepared data sheet for

about six weeks. At the end of the test, which was after 40 days, seedlings were evaluated and final observations were recorded from germination percent.

### Treatment with plant growth regulators (PGRs)

Since increase in germination was not adequate with acid scarified seeds, a set of treatments with GA<sub>3</sub> were tested (Table 1).

### Treatment with 70°C running water

The seeds were treated with 70°C running water for 24, 48, 72 and 96 h. Then the abovementioned stages were performed.

### Combined seed treatments for breaking seed dormancy

In order to increase germination percentage, some of PGR, H<sub>2</sub>SO<sub>4</sub> and 70°C running water treatments were chosen for combination treatments. In this case, GA<sub>3</sub> was treated with H<sub>2</sub>SO<sub>4</sub> and 70°C running water treatment and the seeds that had been treated with 70°C running water were treated with H<sub>2</sub>SO<sub>4</sub> again.

### Statistical analysis

Statistical analysis of data was performed by SAS software. Analysis of significant was assessed by the calculation of the least significant differences at P<0.05.

## RESULTS AND DISCUSSION

The use of various treatments had significant effects on the seed germination in various species (Tables 1 to 4). In this experiment, the results show significant differences among methods used for stimulating *Linum L.* seed germination (P<0.01). Untreated seeds (control) did not germinate. These results suggest that *Linum L.* has an exogenous and endogenous deep dormancy. GA<sub>3</sub> treatment stimulated the germination of four species of *Linum L.* Endogenous GA<sub>3</sub> was widely studied in relation to the breaking of seed dormancy in various species.

The response to GA<sub>3</sub> was dependent on the concentration of GA<sub>3</sub> and a significant difference in germination was observed among seeds treated with various concentrations of GA<sub>3</sub>. At lower concentration (400 ppm), germination was low, but increasing the concentration of GA<sub>3</sub> above 400 ppm significantly improved germination percentage (Table 6). Some studies have also shown that the results of exogenous application of GA<sub>3</sub> on the breaking of seed dormancy and seed germination can differ widely among species and within species (Tigabu and Oden., 2001). Other traits such as days to first germination and days to 50% germination were also affected by GA<sub>3</sub> treatment. Rahman and Park (2000) have found a significant number of *Koelreuteria paniculata* germinated seeds after treatment with GA<sub>3</sub>, but no significant differences in germination was observed among seeds treated with 100, 200 and 300 ppm GA<sub>3</sub>. The highest rate of seed germination among GA<sub>3</sub> treatments in our

**Table 1.** Analysis of deviance for the probability of GA<sub>3</sub> treatments in relation to percent germination, days to first germination and days to 50% germination.

Source	D.F	Mean square								
		Percent germination			Days to first germination			Days to 50% germination		
		400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm
Repetition	3	27.74*	51.74**	4.54 <sup>n.s</sup>	0.58 <sup>n.s</sup>	4.44**	11.58**	1.12 <sup>n.s</sup>	5.91**	22.91**
Medium	1	470**	606**	783**	55.9**	202.1**	41**	59.6**	164.6**	40**
Species	3	117.2**	1515.53**	5339.87**	61.97**	28.02**	89.12**	66.96**	22.33**	122.91**
Treatment	4	3973.91**	7338.55**	15776.65**	748.52**	465.15**	211.37**	1500.8**	1036.03**	729.95**
Medium + species	3	4.08 <sup>n.s</sup>	21.36**	6.6 <sup>n.s</sup>	0.92 <sup>n.s</sup>	8.27**	0.97*	1.06 <sup>n.s</sup>	2.04 <sup>n.s</sup>	0.71 <sup>n.s</sup>
Medium + treatment	4	24.12*	7.96*	29.37**	8.74**	19.05**	2.86**	10.4**	17.5**	3.8*
Species + treatment	12	16.75*	58.58**	334.18	6.95**	4.02**	5.71**	7.4**	3.05**	7.65**
M.T.S	12	2.8 <sup>n.s</sup>	0.0 <sup>n.s</sup>	5.84 <sup>n.s</sup>	0.23 <sup>n.s</sup>	0.36 <sup>n.s</sup>	0.27 <sup>n.s</sup>	1.25 <sup>n.s</sup>	0.0 <sup>n.s</sup>	0.28 <sup>n.s</sup>
Error	-	9.2	3.09	4.8	1.25	0.79	0.37	2.27	0.86	1.47
C.V	-	17.34	6.64	5.03	11.55	11.54	10.36	11.08	8.3	11.7

D.F, Degree of freedom; C.V, coefficient of variance; \*P<0.05; \*\*P<0.01 and n.s, non-significance; M.T.S, medium + treatment + species.

**Table 2.** Analysis of deviance for the probability of 70°C running water in relation to percent germination, days to first germination and days to 50% germination.

Source	D.F	Mean square		
		Percent germination	Days to first germination	Days to 50% germination
Repetition	3	11.09*	6.27**	10.05**
Medium	1	722.5**	10**	104**
Species	3	351.34**	15.6**	8.4**
Treatment	4	5981.9**	221.4**	622.5**
Medium + species	3	47.11**	4.15**	5.35**
Medium + treatment	4	92.35**	3.26*	7.25**
Species + treatment	12	39.94*	0.92 <sup>n.s</sup>	0.93 <sup>n.s</sup>
M.T.S	12	5.91*	0.72 <sup>n.s</sup>	0.5 <sup>n.s</sup>
Error	-	3.92	1.005	0.74
C.V	-	11.3	17.3	9.95

D.F, Degree of freedom; C.V, coefficient of variance; \*P<0.05; \*\*P<0.01 and n.s, non-significance; M.T.S, medium + treatment + species.

**Table 3.** Analysis of deviance for the probability of combination of treatments in relation to percent germination, days to first germination and days to 50% germination.

Source	D.F	Mean square		
		Percent germination	Days to first germination	Days to 50% germination
Repetition	3	45.9**	10.87**	7.09**
Medium	1	1474**	247.5**	563.7**
Species	3	4028.34**	40.53**	25.65**
Treatment	5	18214.008**	258.13**	604.85**
Medium + species	3	28.9**	2.36**	4.46**
Medium + treatment	5	63.18**	10.55**	23.05**
Species + treatment	15	173.77**	2.61**	1.69**
M.T.S	15	6.63 <sup>n.s</sup>	0.27 <sup>n.s</sup>	0.49 <sup>n.s</sup>
Error	-	5.33	0.55	0.64
C.V	-	4.69	11.45	8.2

D.F, Degree of freedom; C.V, coefficient of variance; \*P<0.05; \*\*P<0.01 and n.s, non-significance; M.T.S, medium + treatment + species.

**Table 4.** Analysis of deviance for the probability of sulfuric acid in relation to percent germination, days to first germination and days to 50% germination.

Source	D.F	Mean square					
		Percent germination		Days to first germination		Days to 50% germination	
		25%	40%	25%	40%	25%	40%
Repetition	3	18.37*	27.22**	11.51**	5.75*	1.21 <sup>n.s</sup>	3.41 <sup>n.s</sup>
Medium	1	218.55**	91.5**	99.2**	39**	140.6**	48 <sup>n.s</sup>
Species	3	9426.65**	253.22**	120.3**	73.25**	138.38**	82.08**
Treatment	4	6924.48**	1127.98**	328.009**	507.05**	896.25**	1138.3**
Medium + species	3	10.72 <sup>n.s</sup>	0.98 <sup>n.s</sup>	0.69 <sup>n.s</sup>	0.98 <sup>n.s</sup>	12.57**	6.51*
Medium + treatment	4	16.35*	6.2 <sup>n.s</sup>	5.95**	3.1**	9.6**	5.47*
Species + treatment	12	715.23**	22.69**	9.01**	4.71**	9.35**	6.46**
M.T.S	12	2.46 <sup>n.s</sup>	0.25 <sup>n.s</sup>	0.446 <sup>n.s</sup>	0.33 <sup>n.s</sup>	1.11 <sup>n.s</sup>	2.07 <sup>n.s</sup>
Error	-	5.27	4.79	0.86	0.8	1.05	2.69
C.V	-	9.48	21.03	14.43	11.29	10.01	14.21

D.F, Degree of freedom; C.V, coefficient of variance; \*P<0.05; \*\*P<0.01 and n.s, non-significance; M.T.S, medium + treatment + species.

**Table 5.** Comparison of averages of the effects of species on the percent germination, days to first germination and days to 50% germination in relation to GA<sub>3</sub> treatments.

Species	Percent germination			Days to first germination			Days to 50% germination		
	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm
<i>L. austriacum</i>	18.82 <sup>A</sup>	33.71 <sup>A</sup>	52.7 <sup>A</sup>	8.25 <sup>C</sup>	6.69 <sup>C</sup>	5.27 <sup>B</sup>	13.46 <sup>B</sup>	10.58 <sup>C</sup>	8.97 <sup>C</sup>
<i>L. mucronatum</i>	18.6 <sup>A</sup>	19.67 <sup>D</sup>	28.17 <sup>C</sup>	10.8 <sup>A</sup>	7.67 <sup>B</sup>	8.15 <sup>A</sup>	15.35 <sup>A</sup>	12.2 <sup>A</sup>	12.9 <sup>A</sup>
<i>L. nervosum</i>	17.47 <sup>A</sup>	29.2 <sup>B</sup>	25.37 <sup>A</sup>	9.02 <sup>B</sup>	7.82 <sup>B</sup>	5.25 <sup>B</sup>	12.22 <sup>C</sup>	10.65 <sup>C</sup>	9.8 <sup>B</sup>
<i>L. album</i>	15.07 <sup>B</sup>	23.5 <sup>C</sup>	41.9 <sup>B</sup>	10.6 <sup>A</sup>	8.75 <sup>A</sup>	5 <sup>B</sup>	13.37 <sup>B</sup>	11.3 <sup>B</sup>	9.62 <sup>B</sup>

A, B, C, Grouping of comparison of averages on Duncan's test.

**Table 6.** Comparison of averages of the effects of treatments on the percent germination, days to first germination and days to 50% germination in relation to GA<sub>3</sub> treatments.

Treatment	Percent germination			Days to first germination			Days to 50% germination		
	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm
T <sub>0</sub> : Control	1.81 <sup>E</sup>	1 <sup>E</sup>	1 <sup>E</sup>	1.28 <sup>E</sup>	1 <sup>D</sup>	1 <sup>C</sup>	1.28 <sup>E</sup>	1 <sup>E</sup>	1 <sup>D</sup>
T <sub>1</sub> : 72 h	10.62 <sup>D</sup>	23.31 <sup>D</sup>	44.43 <sup>D</sup>	13.34 <sup>A</sup>	10.43 <sup>A</sup>	8.21 <sup>A</sup>	18 <sup>A</sup>	14.93 <sup>A</sup>	14 <sup>A</sup>
T <sub>2</sub> : 120 h	21.12 <sup>C</sup>	32.68 <sup>C</sup>	53.84 <sup>C</sup>	12.12 <sup>B</sup>	10 <sup>A</sup>	7.59 <sup>A</sup>	17.09 <sup>B</sup>	14.34 <sup>B</sup>	13.4 <sup>AB</sup>
T <sub>3</sub> : 144 h	24.83 <sup>B</sup>	35.59 <sup>B</sup>	57.59 <sup>B</sup>	11.51 <sup>C</sup>	9.03 <sup>B</sup>	6.75 <sup>B</sup>	16.29 <sup>C</sup>	13.18 <sup>C</sup>	12.18 <sup>BC</sup>
T <sub>4</sub> : 168 h	29.25 <sup>A</sup>	39 <sup>A</sup>	61.54 <sup>A</sup>	10.28 <sup>D</sup>	8.03 <sup>C</sup>	6.06 <sup>B</sup>	15.21 <sup>D</sup>	12.15 <sup>D</sup>	11.09 <sup>C</sup>

A, B, C, D, E, Grouping of comparison of averages on Duncan's test.

**Table 7.** Comparison of averages of the effects of treatments on the percent germination, days to first germination and days to 50% germination in relation to sulfuric acid treatments.

Treatment	Percent germination		Days to first germination		Days to 50% germination	
	25%	40%	25%	40%	25%	40%
T <sub>0</sub> : Control	0.96 <sup>E</sup>	1 <sup>E</sup>	0.96 <sup>E</sup>	1 <sup>E</sup>	0.98 <sup>E</sup>	1 <sup>E</sup>
T <sub>1</sub> : 10 min	20.03 <sup>D</sup>	9.28 <sup>D</sup>	9.12 <sup>A</sup>	10.81 <sup>A</sup>	13.78 <sup>A</sup>	15.59 <sup>A</sup>
T <sub>2</sub> : 15 min	27.93 <sup>C</sup>	11.28 <sup>C</sup>	8.12 <sup>B</sup>	10.06 <sup>B</sup>	13.09 <sup>B</sup>	14.06 <sup>B</sup>
T <sub>3</sub> : 20 min	33.59 <sup>B</sup>	13.84 <sup>B</sup>	7.43 <sup>C</sup>	9.4 <sup>C</sup>	12.37 <sup>C</sup>	13.96 <sup>B</sup>
T <sub>4</sub> : 25 min	38.56 <sup>A</sup>	16.62 <sup>A</sup>	6.59 <sup>D</sup>	8.5 <sup>D</sup>	11.15 <sup>D</sup>	13.12 <sup>C</sup>

A, B, C, D, E, Grouping of comparison of averages on Duncan's test.

study was 61.54% using 1600 ppm concentration for 168 h in *L. mucronatum* and the lowest rate of that was 10.62% using 400 ppm concentration for period of 24 h in *L. album*. Washing and moist chilling are standard techniques which have been used for dormant seeds of many species to enhance the germination of dormant seeds and reduce endogenous dormancy successfully (ISTA, 1996). Furthermore, the results of our experiments show that treatment with sulfuric acid gave the best result when a concentration of 25% was applied for 25 min. In this case, the time period of treatment has a more prominent role on seed germination than the acid concentration (Table 7).

Although the promising results of the seeds soaking of *Teucrium polium* species in water for 72 h has been proven in previous experiments (Khoosheki and Azizi, 2006; Najdafi et al., 2006), however, the use of this method has not been beneficial in our experiment due to the presence of mucilage on the seed coat which was acting as a physical barrier. In our experiment, this barrier was overcome by rinsing the seeds in water together with the use of water temperature at 70°C for the breakdown of seed dormancy (Table 9). In order to increase the effectiveness and speed of seed germination, a combination of treatments was also used. These treatment combinations consisted of GA<sub>3</sub> together with H<sub>2</sub>SO<sub>4</sub> treatment and H<sub>2</sub>SO<sub>4</sub> treatment together with rinsing with 70°C water and finally GA<sub>3</sub> together with water rinsing. Based on the obtained results, if the seeds were first

treated together with GA<sub>3</sub> and followed by H<sub>2</sub>SO<sub>4</sub> treatment, no successful results would be obtained and the level of seed germination will not be significant. However, when seeds were firstly treated with a 10% solution of H<sub>2</sub>SO<sub>4</sub> followed by 168 h of GA<sub>3</sub>, successful results were obtained (Table 10). Moreover, the results also show that the use of acid caused the removal of mucilage and seed coat as the first barriers for seed germination and then cold treatment caused a reduction in abscisic acid production in the embryo and increasing of the potential and speed in seed germination. Basically, the abscisic acid through the induction of hormones such as GA<sub>3</sub> causes seed dormancy. Comparing the average that was performed by Duncan test, there were high levels of variation between species and treatments and the similar letters is the sign of significant differences between groups (Tables 5 to 8).

Results of our experiment indicate that in all of the four species of *Linum*, the percentage and speed of germination was higher in water + agar medium as compared with the MS medium. It appears that the minerals within the MS medium and probably its pH (5.8) has acted as an effective barrier and was caused to reduce the percent of seed germination (Table 11). When the interaction between the growth medium and species was analyzed, except to a few instances, no significant effect was observed as an indication. Considering the fact that *Linum* species grow at altitudes of 1300 to 2000 m and in light and wallet leached soils with a minimum of nutrient,

**Table 8.** Comparison of averages of the effects of species on the percent germination, days to first germination and days to 50% germination in relation to 70°C running water treatment (a) and combination treatments (b), sulfuric acid treatments (c).

Species	Percent germination				Days to first germination				Days to 50% germination			
	a	b	c		a	b	c		a	b	c	
			0.25	0.40			0.25	0.40			0.25	0.40
<i>L. austriacum</i>	8.5 <sup>B</sup>	55.08 <sup>B</sup>	36.05 <sup>B</sup>	12.8 <sup>A</sup>	19.52 <sup>A</sup>	5.27 <sup>D</sup>	5.52 <sup>B</sup>	6.67 <sup>B</sup>	5.07 <sup>B</sup>	8.81 <sup>D</sup>	9.2 <sup>D</sup>	10 <sup>B</sup>
<i>L. mucronatum</i>	9.17 <sup>A</sup>	36.41 <sup>D</sup>	10.85 <sup>C</sup>	7.87 <sup>B</sup>	14.82 <sup>B</sup>	7.47 <sup>A</sup>	7.72 <sup>A</sup>	9.17 <sup>A</sup>	6.32 <sup>A</sup>	10.52 <sup>A</sup>	11.57 <sup>B</sup>	12.47 <sup>A</sup>
<i>L. nervosum</i>	8.07 <sup>C</sup>	56.52 <sup>A</sup>	38.9 <sup>A</sup>	12.32 <sup>A</sup>	20.35 <sup>A</sup>	6.75 <sup>B</sup>	4.47 <sup>C</sup>	6.9 <sup>B</sup>	5.45 <sup>B</sup>	9.5 <sup>C</sup>	8.22 <sup>D</sup>	10.7 <sup>B</sup>
<i>L. album</i>	8.77 <sup>B</sup>	48.81 <sup>C</sup>	11.07 <sup>C</sup>	8.62 <sup>B</sup>	14.85 <sup>B</sup>	6.54 <sup>B</sup>	8.07 <sup>A</sup>	9.07 <sup>A</sup>	6.3 <sup>A</sup>	10.02 <sup>B</sup>	12.1 <sup>A</sup>	13.02 <sup>A</sup>

A, B, C, D: Grouping of comparison of averages on Duncan's test.

**Table 9.** Comparison of averages of the effects of treatments on the percent germination, days to first germination and days to 50% germination in relation to 70°C running water treatment.

Treatment	Percent germination	Days to first germination	Days to 50% germination
T <sub>0</sub> : Control	1 <sup>E</sup>	1.31 <sup>E</sup>	1 <sup>E</sup>
T <sub>1</sub> : 24 h	8.6 <sup>D</sup>	7.9 <sup>A</sup>	12.03 <sup>A</sup>
T <sub>2</sub> : 48 h	16.7 <sup>C</sup>	7.37 <sup>B</sup>	11 <sup>B</sup>
T <sub>3</sub> : 72 h	24.43 <sup>B</sup>	6.59 <sup>C</sup>	10.12 <sup>C</sup>
T <sub>4</sub> : 96 h	36.15 <sup>A</sup>	5.75 <sup>D</sup>	9.06 <sup>D</sup>

A, B, C, D, Grouping of comparison of averages on Duncan's test.

**Table 10.** Comparison of averages of the effects of treatments on the percent germination, days to first germination and days to 50% germination in relation to combination of treatments.

Treatment	Percent germination	Days to first germination	Days to 50% germination
T <sub>0</sub> : Control	1 <sup>E</sup>	1 <sup>E</sup>	1 <sup>E</sup>
T <sub>1</sub> : GA <sub>3</sub> (168 h and 1600 ppm) in 4°C	57.34 <sup>C</sup>	8.68 <sup>C</sup>	12.62 <sup>A</sup>
T <sub>2</sub> : T <sub>1</sub> + sulfuric acid 10%	60.4 <sup>B</sup>	7.59 <sup>C</sup>	11.56 <sup>B</sup>
T <sub>3</sub> : Sulfuric acid 10% + T <sub>1</sub>	64.28 <sup>A</sup>	6.06 <sup>D</sup>	10.18 <sup>D</sup>
T <sub>4</sub> : T <sub>1</sub> + 70°C flowing water	58.09 <sup>C</sup>	7.53 <sup>C</sup>	11 <sup>C</sup>
T <sub>4</sub> : 70°C flowing water + sulfuric acid	54.12 <sup>D</sup>	8.18 <sup>B</sup>	11.9 <sup>B</sup>

A, B, C, D, E, Grouping of comparison of averages on Duncan's test.

therefore, in seed germination studies, better results was obtained when a minimum of nutrients was present in the growth medium as it was in the medium of water + agar. Based on the results as shown in Tables 9 to 12, it is obvious that in most instances, interaction exists between the type of treatment and species. This is an indication that a specific species in combination with a specific treatment give a better germination result that cannot be observed with other treatments. For example, the combination treatment of T<sub>3</sub> and *L. mucronatum* showed the best performance. On the other hand, in the treatment of running water with *L. austriacum* and *L. nervosum* the best result has been obtained. Similarly in

the treatment of GA<sub>3</sub>, the concentration of 1600 ppm for 144 h, the best result has been obtained for the *L. mucronatum* species, whereas the treatment for the *L. album* species has not been promising. Meanwhile, the album species had performed better with 1600 ppm concentration for 196 h.

## Conclusion

Although the MS medium is suitable for the seed germination of most plant species, the *Linum* species in this project did not act well in this growth medium. The best treatment for seed germination was the combination of

**Table 11.** Comparison of averages of the effects of media on the percent germination, days to first germination and days to 50% germination in relation to 70°C running water treatment (a), combination treatments (b) and sulfuric acid treatments (c).

Medium	Percent germination				Days to first germination				Days to 50% germination			
	a	b	c		a	b	c		a	b	c	
			0.25	0.40			0.25	0.40			0.25	0.40
Water + Agar	19.51 <sup>A</sup>	51.97 <sup>A</sup>	24.38 <sup>A</sup>	11.16 <sup>A</sup>	5.53 <sup>B</sup>	5.37 <sup>B</sup>	5.66 <sup>B</sup>	7.46 <sup>B</sup>	7.83 <sup>B</sup>	8 <sup>B</sup>	9.33 <sup>B</sup>	11 <sup>B</sup>
MS growth medium	15.26 <sup>B</sup>	46.43 <sup>B</sup>	23.05 <sup>B</sup>	9.65 <sup>B</sup>	6.03 <sup>A</sup>	7.64 <sup>A</sup>	7.23 <sup>A</sup>	8.45 <sup>A</sup>	9.45 <sup>A</sup>	11.42 <sup>A</sup>	11.21 <sup>A</sup>	12.10 <sup>A</sup>

A, B: Grouping of comparison of averages on Duncan's test.

**Table 12.** Comparison of averages of the effect of media on the percent germination, days to first germination and days to 50% germination in relation to GA<sub>3</sub> treatment.

Medium	Percent germination			Days to first germination			Days to 50% germination		
	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm	400 ppm	800 ppm	1600 ppm
Water + agar	11.16 <sup>A</sup>	28.44 <sup>A</sup>	46 <sup>A</sup>	8.45 <sup>A</sup>	6.6 <sup>B</sup>	5.41 <sup>B</sup>	12.1 <sup>A</sup>	10.16 <sup>B</sup>	9.8 <sup>B</sup>
MS growth medium	9.6 <sup>B</sup>	24.53 <sup>B</sup>	41.57 <sup>B</sup>	7.46 <sup>B</sup>	8.8 <sup>A</sup>	6.42 <sup>A</sup>	11 <sup>B</sup>	12.2 <sup>A</sup>	10.8 <sup>A</sup>

A, B, Grouping of comparison of averages on Duncan's test.

acid + GA<sub>3</sub>.

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