

**Data on Scarification and Stratification Treatments on Germination and Seedling Growth of *Ziziphus Jujuba* Seeds****S. Karimpour, G.H. Davarynejad, H. Rouhbakhsh, E. Ardakani***Department of Horticultural Science, Ferdowsi University of Mashhad, Mashhad, Iran.*S. Karimpour, G.H. Davarynejad, H. Rouhbakhsh, E. Ardakani; Data on Scarification and Stratification Treatments on Germination and Seedling Growth of *Ziziphus Jujuba* Seeds**ABSTRACT**

*In vitro* and *in vivo* culture of *Z. jujuba* seeds under stratification and scarification were carried out. Sulfuric acid 98% ( $H_2SO_4$ ) exposure time (0, 5, 10 and 15 minutes), stratification and scarification (acid scarification; 0, 5, 10 and 15 minutes and mechanical scarification) were used for *in vitro* and *in vivo* conditions, respectively. In *in vitro* culture, Germination percentage, vigor index and the rootlet number increased by ascending acid exposure time while the highest of seedling length was obtained in 5 minutes acid exposure. In *in vivo* culture, stratification led to decrease of seedling and radicle length and mechanical scarification raised germination percentage, vigor index, seedling length, radicle length, rootlet number and rootlet length. Stratification plus mechanical scarification had the highest the rootlet number and length whereas other characteristics had the highest amount on mechanical scarification without stratification in the stratification  $\times$  scarification interactions. No germination occurred in 15 minute acid exposure *in vivo* culture.

**Key words:** Jujuba, Moist chilling, Physical dormancy, Seed germination.**Introduction**

The *Ziziphus* genus with 135-170 species systematically was placed in Rhamnaceae family [3]. Five species of *Ziziphus* namely *Z. nummularia* (Burm. f.), *Z. spina-christi*, *Z. jujuba*, *Z. oxyphylla*, *Z. aucherii* grew in Iran. *Z. jujuba* is commercially important because of cosmetic and pharmaceutical uses. The trees are deciduous and tolerant to drought and it is able to grow in many types of soils with high salinity or alkalinity [2]. Drupe fruits are formed of red exocarp, edible fleshy mesocarp, and stony endocarp with 1-2 seeds.

Poor germination of *Ziziphus* seeds seems caused by dormancy, hard woody endocarp and even seed coat that covering around the seeds. The existence of these Barriers is useful for its survival, but on the other hand it is an undesirable characteristic for growers. Hard woody endocarp and also seed coat increase the time of achievement to final germination percentage. Maraghi *et al.* [6] achieved 100% germination in *Z. lotus* seeds when endocarps of fruits artificially were broken. According Baskin and Baskin [7,8] comprehensive classification System, Finch-Savage and Leubner-Metzger [10] were reported physiological, physical, physiological-physical and non-dormancy in Seeds of Rhamnaceae family. Non-dormancy was seen in a few species of *Ziziphus* [7]. Pareek [9] proposed applying of

mechanical scarification and non-mechanical methods for seed broken dormancy of *Ziziphus* species to be effective.

Cold stratification are used for physiological and embryo dormancy while mechanical and chemical scarification are useful methods for physical ones. Olmez *et al.* [4] found the durations of cold stratification are insufficient to overcome seed dormancy of *Z. jujuba*. They also stated the greenhouse condition were more effective on seed germination over open field conditions. In addition to hard woody endocarp, it seems the seed coat delays or decreases seed germination in this species. So, this study was conducted to investigate the effect of stratification and scarification on seed germination and seedling growth of *Z. jujuba* seed in *in vitro* and *in vivo* conditions.

**Materials and Methods**

*Z. jujuba* seeds were collected in May 2012 from trees growing in *in vivo* condition of Khaf county (34°30' N, 60°00' E, 970 m altitude) of Razavi Khorasan Province, Iran. We used seeds in fully ripe stage when the exocarp has turned red. In both experiments exocarp, mesocarp and hard woody exocarp were removed and seed with brown seed coat were used.

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*First experiment: in vitro germination:*

Seeds after disinfectant with 1% sodium hypochlorite (NaOCl) supplemented with Tween-20 (0.2%) for 10 minutes were cleaned with sterile distilled water. Then, seeds were treated with Sulfuric acid 98% (H<sub>2</sub>SO<sub>4</sub>) for 0 (as control), 5, 10 and 15 minute. Seeds were washed with sterile distilled water 3 times and were cultured in vials (100 ml) containing 20 ml medium (8 gL<sup>-1</sup> agar – agar, Merck®). The cultures were grown at 24 ± 1 °C in a 16-h photoperiod at light intensity of 40 μmol m<sup>-2</sup>s<sup>-1</sup> provided by white fluorescent tubes.

*Second experiment: in vivo germination:*

In this experiment we used two groups of seeds: stratificated (30 days at 4 °C in wet sand) and unstratified seeds. Seeds were surface sterilized using 1% sodium hypochlorite (NaOCl) for 10 minutes, and washed three times with distilled water. Consequently, Seeds of both groups treated by acid scarification, Sulfuric acid 98% (H<sub>2</sub>SO<sub>4</sub>), for 0 (as control), 5, 10 and 15 minute and mechanical scarification and they were cleaned with distilled water for 3 times. In mechanical scarification, two incisions on either side of the seed coat were created by a sharp scalpel blade. Seeds were cultured in pots with sterile peat moss medium. Pots were maintained in laboratory conditions. Germination percentage (%), vigor index, seedling length (mm), radicle length (mm), the number of rootlets, rootlet length (mm) after 5 and 7 days were recorded for first and second experiment, respectively. Vigor index was calculated according to germination percentage × seedling total length [1].

*Statistical analysis:*

First experiment was performed in completely randomized design with 5 replication containing 10 seeds. Second experiment was carried out in factorial (stratification and scarification) in a completely randomized design with 3 replication containing 20 seeds. Germination percentage was transformed to arcsine before analysis. Data were analyzed by SAS PROC GLM [14] and mean values were compared according to Least Significant Difference (LSD) test at 5% probability.

*Results:**First experiment: In vitro germination:*

ANOVA indicated that germination percentage, seedling length ( $p \leq 0.05$ ), vigor index, the rootlet number ( $p \leq 0.01$ ) were affected by acid exposure time while radicle and rootlet length was not affected (Table 1).

The highest of germination percentage was obtained in 15 and 10 minute acid exposure with

72% and 66% and the lowest was showed in 0 and 5 with 39% and 42%, respectively (Fig. 1 - a). With increasing of acid exposure time, seed coat dissolves more and becomes thinner that make germination easier. It was observed the highest vigor index in 15 minute acid exposure. The high vigor index in this treatment is because of high germination percentage (Fig. 1 - b).

In 5 minute acid exposure the highest of seedling length (81.23 mm) was showed and in 10 and 15 minute were decreased. Do not use acid induced lowest of seedling length (52.06 mm) (Fig. 1 - c). With increasing acid exposure time the rootlet numbers increased. The highest of the rootlet number (7.734 - 5.860) and the lowest (0.668) were observed in 15 and 10 minute acid exposure and non- acid exposure, respectively (Fig. 1 - d).

*Second experiment: In vivo germination:*

ANOVA indicated that all characteristics were affected by scarification and stratification × scarification interaction ( $p \leq 0.01$ ), seedling and radicle length were affected by stratification ( $p \leq 0.05$ ), too. Rootlet length only was affected by scarification and stratification × scarification interaction ( $p \leq 0.05$ ) (Table 2).

The highest of germination percentage (57.50%) was observed in mechanical scarification and with increasing acid exposure time decreased that the lowest of germination percentage (00.00%) was showed in acid scarification at 15 minute exposure time (Fig. 3 - a). Mechanical scarification without stratification induced the highest germination percentage (70.00%) in stratification × scarification interaction treatments (Table 3). Mechanical scarification induced the highest vigor index, it was indicated a decreasing process with increasing acid exposure time similar to germination percentage (Fig. 3 - b). Mechanical scarified seeds with unstratification had the higher vigor index (Table 3).

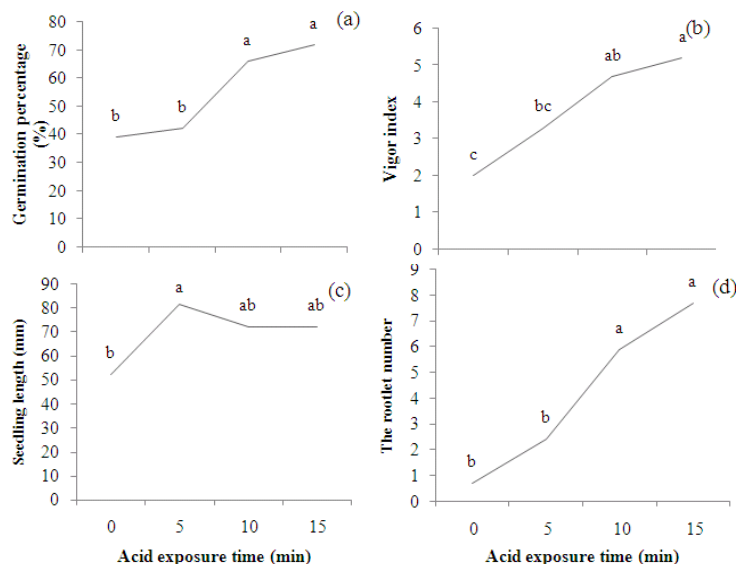
Unstratified seeds produced higher seedlings (90.60 mm) rather seeds were treated with stratification (61.24 mm) (Fig. 2). Stratification is a standard way to enhance the germination rate in some dormant seeds. Mechanical scarification led to the highest seedling length (210.72 mm) and we were a decreasing in seedling length when the time of exposure increased so that 74.39 mm to 0.00 mm in 0 reached in 15 minute acid exposure (Fig. 3 - c). Mechanical scarification without stratification led to more seedling growth (264.00 mm, Table 3).

Similar to seedling length, radicle length was affected by stratification and the stratification had a lowering effect on it that inhibited radicle growth. Radicle in seeds treated with mechanical scarification had higher growth (106.500 mm) and a decreasing trend was recorded from 0 up to 15 minute acid exposure from 21.388 mm to 0.000 mm

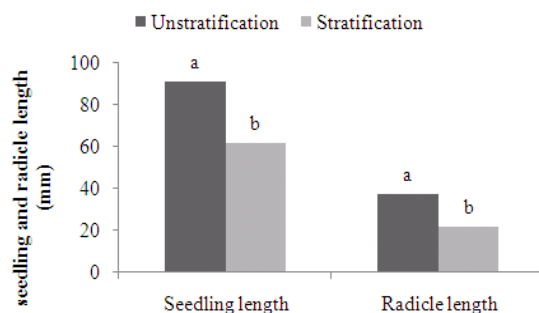
(Fig. 3 - d). Radicle growth had higher amount (148.33) in mechanical scarified seeds that did not treated with stratification (Table 3).

Mechanical scarified seeds produced the highest rootlet number (2.712) and rootlet length (4.810 mm)

(Fig. 3 - e and f). In contrast of above characteristics the rootlet number and rootlet length have the highest amount in seeds treated with stratification and mechanical scarification with 3.443 and 8.620 mm, respectively (Table 3).



**Fig. 1:** The effect of different acid exposure time on germination percentage (a), vigor index (b), seedling length (c) and the rootlet number (d) in *Z. jujuba* seeds in *in vitro* condition. The same letters in columns are not significantly different by LSD's test at 5%.



**Fig. 2:** Unstratification and stratification effect on seedling and radicle length of *Z. jujuba* *in vivo* condition.

**Table 1:** ANOVA of different acid exposure time on germination and some seedling growth characteristics of *Z. jujuba* seeds *in vitro* condition.

Sources	df	Mean Square					
		Germination percentage	Vigor index	Seedling length	Radicle length	The rootlet number	Rootlet length
Acid scarification	3	1391.25 *	10.36 **	753.543 *	101.650 <sup>ns</sup>	51.579 **	3.117 <sup>ns</sup>
Error	16	281.25	1.76	258.501	99.572	2.808	9.036
Corrected Total	19						

\*\*: significant at 0.01, \*: significant at 0.05 and <sup>ns</sup>: not significant.

**Table 2:** ANOVA of different acid exposure time on germination and some seedling growth characteristics of *Z. jujuba* seeds *in vivo* condition.

Sources	df	Mean Square					
		Germination percentage	Vigor index	Seedling length	Radicle length	The rootlet number	Rootlet length
Stratification	1	120.00 <sup>ns</sup>	24.76 <sup>ns</sup>	6463.017 *	1820.056 *	0.449 <sup>ns</sup>	24.752 <sup>ns</sup>
Scarification	4	3011.25 **	173.06 **	40519.791 **	11581.578 **	7.540 **	23.036 <sup>ns</sup>
Stratification × Scarification	4	1563.75 **	71.97 **	19786.662 **	3167.094 **	6.919 **	33.888 *
Error	20	307.50	13.39	1036.810	272.369	1.526	11.538
Corrected Total	29						

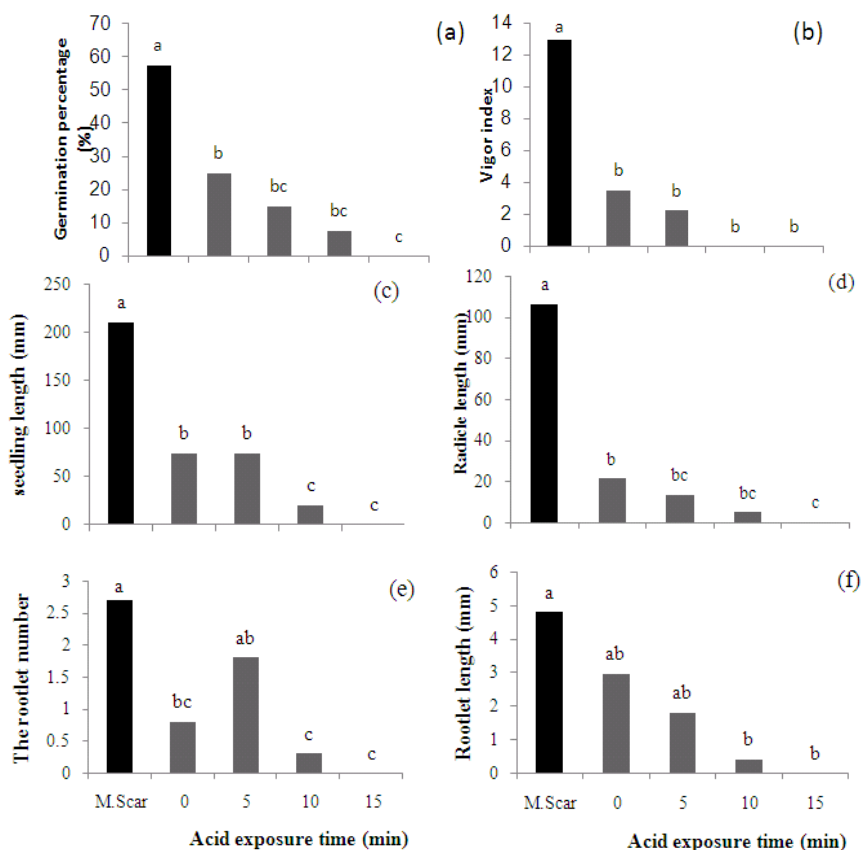
\*\*: significant at 0.01, \*: significant at 0.05 and <sup>ns</sup>: not significant.

**Table 3:** The stratification × scarification interaction effect on germination percentage, vigor index, seedling length, radicle length, the rootlet number and rootlet length of *Z. jujuba* in *in vivo* condition.

Treatments		Germination percentage (%)		Vigor index		Seedling length (mm)		Radicle length (mm)		The rootlet number		Rootlet length (mm)	
		UST <sup>†</sup>	ST	UST	ST	UST	ST	UST	ST	UST	ST	UST	ST
Mechanical scarification		70.00 a <sup>*</sup>	45.00 ab	18.8 a	7.0 b	264.00 a	157.45 b	148.33 a	64.67 b	2.0 ab	3.443 a	1.00 bc	8.62 a
Acid exposure time (minute)	0 (control)	00.00 d	50.00 ab	0.0 c	7.1 b	0.00 c	148.78 b	0.00 d	42.78 bc	0.0 b	1.7 ab	0.00 c	5.92 ab
	5	30.00 bc	00.00 d	4.4 cb	0.0 c	148.00 b	0.00 c	27.00 cd	0.00 d	3.7 a	0.0 b	3.62 abc	0.00 c
	10	15.00 d	00.00 d	0.0 c	0.0 c	41.00 c	0.00 c	10.00 d	0.00 d	0.7 b	0.0 b	0.83 bc	0.00 c
	15	00.00 d	00.00 d	0.0 c	0.0 c	0.00 c	0.00 c	0.00 d	0.00 d	0.0 b	0.0 b	0.00 c	0.00 c

<sup>†</sup>UST: unstratification and ST: stratification.

<sup>\*</sup>Means followed by the same letter in columns are not significantly different by LSD's test at 0.05 probably level.

**Fig. 3:** The mechanical scarification (M.Scar) and acid exposure time effects on germination percentage (a), vigor index (b), seedling length (c), radicle length (d), the rootlet number (e) and rootlet length (f) of *Z. jujuba* in *in vivo* condition. The same letters in columns are not significantly different by LSD's test at 5%.

#### Discussion:

In *in vitro* condition it was observed an increase in germination percentage with acid exposure time enhancing result of more seed coat breakup, while in *in vivo* we showed decreasing in it. Because of the existence more suitable growth and sterile conditions in *in vitro*, more elimination of seed coat, as protective vegetative points, led to more germination, whereas the protective seed coat is needful for better germination in unsterile *in vivo* condition. Therefore, germination ranges were 39 - 72% and 0 - 25% in *in vitro* and *in vivo*, respectively. Despite the low germination percentage in *in vivo* condition, mechanical scarification on unstratified seeds had the highest of germination (70%).

Stratification was used for elimination of embryo requirements in order to improve germination [11,12,13] in our study; it was found

stratification had no effect on germination percentage, vigor index, the rootlet number and length. Mechanical scarification improved germination and seedling growth characteristics, so it can be state that *Z. jujuba* has physical dormancy that avoided water absorption not physiological dormancy. Since the absorption of water by seed coat was occurred in stratification, the seed coat was more susceptible to acid exposure time, that it is enhanced seed survival risks. Consequently, by increasing acid exposure time we showed a decrease in seedling and radicle length in stratified seeds. Stratified seeds in compared with control enhanced germination percentage from 0% up to 50%.

Superiority of mechanical scarification treatment over the other treatments was quite evident in both stratification and unstratification. Stratification is useful when acid stratification was not used and the higher acid exposure time of 5 minute was not

suggested. As noted above, we can observe stratification effect on improving of seed coat water absorption. 14.1% germination was reported for *Z. jujuba* seeds that were 20 days stratified and greenhouse condition cultured [4] while Lyrene [5] recommended H<sub>2</sub>SO<sub>4</sub> scarification (120 - 360 minute) and stratification (5 °C) for 60 to 90 days.

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