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Scarification in Acetic Acid, Hot Water, and Cold Sand Stratification to Improve Carob (*Ceratonia Siliqua* L.) Seed Germination

ABSTRACT

This study was carried out to determine the effects of acetic acid, hot water, and cold sand stratification scarification at different periods on seed germination of carob (*Ceratonia siliqua* L.) in Salah Alden province. The seeds were collected from Kirkuk province area on September 2016. Carob seeds stratified and not stratified treated by soaking in glacial acetic acid for 20, 40, 60, and 120 min. at lab temperature (25°C). Hot water (100°C) also used for soaking the seeds for 20, 40, 60, and 120 min. Experimental design of Randomized Complete Block Design (RCBD) with 3 replicates, and 10 seeds per each treatment per each replicate were used and the results were compared to non-treated seeds. After soaking the seeds in acetic acid and hot water, they washed thoroughly and sowed in black polyethylene grow bags. The soil used was loamy with added organic matter. After 7 months, data recorded on the germination percent and growth features including plant height, leaf area, total chlorophyll, root length, fresh and dry weight of vegetative parts, nitrogen, phosphorous, and potassium percentages in the plant. The results showed that the treatment with hot water for 60 minutes achieved the highest percent of germination (96.67%), highest leaf area 12.86 cm², highest root dry weight (0.14 gm.), and nitrogen percent of 5.52%. The treatment for 40 minutes with hot water gave the highest fresh weight of vegetative parts was 18.6 gm. and root fresh weight (7.05 gm.). The treatment with hot water for 120 minutes achieved the highest percent of phosphorous and potassium (0.49% and 2.07% respectively).

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INTRODUCTION

Carob (*Ceratonia siliqua* L.) tree grows in the Mediterranean basin area where the soils and the environmental conditions are suitable for the tree growth. Carob tree is an evergreen tree. The fruits are pods of light brown color and long contain seeds.

The pods contain 55% sugar, 15% high quality proteins, and 6% fat. In addition to these compounds, there are some vitamins as A, B1, B2, and D. Some of elements as potassium, calcium, iron, phosphorus, manganese, barium, copper, nickel, and magnesium. While, the seeds contain 60% Proteins. Both pods and seeds are used in food and pharmaceutical industries (Loeb et al., 1989, and Gruendel et al., 2006). Carob tree serves as ornamental tree and roadside tree as the tree helps protection of soil from erosion and as fence against sand and some storms.

Carob tree seeds with hard coat and it is impermeable to water. In addition to that, carob tree seeds pass through some physiological dormancy based on the environmental conditions where the tree lives in. APAT (2003) reported that average germination of carob seeds was 60-95% after treatment

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because of tough and impermeable seed coat that impede water absorption, thus hindering germination. Without any treatment, germination percentage rarely exceeds 10%. The treatments: Scarification, mechanical with corrosive material, soaking in boiled water for 12-24 hours, acid as 90% H₂SO₄ for 20 min. and even some alkalis. The natural germination of the seeds is lower than 10% (Piotto and Di Noi, 2003). Since the tree is growing in many areas in the Mediterranean area, sometimes farm animals eat the pods, the seeds undergo some exposure to acids and digestion enzymes in the stomach of those animals, and they grow naturally better, El-Shatnawi et al. (2001). This fact withdrew the attention of researchers to apply some artificial pre-sowing treatments to enhance the seed germination. Those treatments include mechanical scarification, soaking in hot/cold water, soaking in mineral acids such as hydrochloric, sulfuric, nitric, and phosphoric acid. Some plant hormones also used to do so such as gibberellin. Concentrated (98%), Sulfuric acid was used to treat the seeds for 30 minutes and the germination percent was raised to 93%, (Gubbuk, 2009). Concentrated sulfuric acid (96%) was also used for 30, minutes and gave the germination of 88.86%. Warm (10, 15, 20, and 25°C) water was applied for 8-16 hours, the germination increased from 23.28% (control treatment), of non-treated seeds to 88.90% of treated seeds, (Perez-Garcia, 2009). Zaen El-Deen, et al., (2014), Studied the treatment of carob seed for germination with dipping in H₂SO₄ of 60% for 30 min., soaking in warm water 30°C for 72 hours, hot water for 10 min., Dipping in GA3 (25 mg/l) for 24 hours, and dipping in acetone 80%. They found that the highest germination percentage and rate, highest plant length, number of leaves/plant, root length, and dry weight were with 60% H₂SO₄. Sulfuric acid (95%), compared to gibberellic acid (1000 ppm and 1500 ppm), the germination percent was different as for sulfuric acid was 90.88% while for gibberellic acid was 28.90%, (Boston and Kilic, 2014). Martins (1996), reported an increase of the germination percent to 86.7%, of carob tree seeds when treated with sulfuric acid of 98% concentration for 15 minutes, compared to 78% germination percent with hot water (90°C), treatment. Carob tree seeds germination improved to 95.69% by mechanical scarification and making some cracks on the seed coat after concentrated sulfuric acid treatment, (Karaguzel et al., 2002). The main goals of this study were to find out the best treatment of acetic acid or hot water with/without stratification on carob tree seeds germination percent. Also to compare the treatments effect on vegetative growth of the produced seedlings.

MATERIALS AND METHODS

The experiment was carried out at the wooden canopy and greenhouse/Horticulture and Gardening Landscape Department /College of Agriculture/University of Tikrit from October 2, 2016 to May 2, 2017, to study the effect of scarification in acetic acid, hot water soaking and cold sand stratification on carob tree (*Ceratonia siliqua* L.) seed germination. The seeds were collected on September 10, 2016 from 20-year old trees at Kirkuk Technical Institute gardens. The soil was mixture of loamy textured with addition of small amount of organic matter. The soil mixture put in polyethylene black colored grow bags (10 cm diameter and 5 kg. capacity). Glacial acetic acid and hot water used for soaking the stratified and non-stratified seeds for 0, 20, 40, 60, and 120 minutes. Hot water that used in the experiment as the water brought to boiling point then the seeds were soaked in and the temperature kept adjusted higher than 80°C in the oven. The ratio of liquid: seeds was 3:1 and measured by graduated cylinder. The following simples represented the treatments:

- | | |
|---|-----------------------------|
| T1: No treatment (0.00 min. - control) | T2: Acetic acid for 20 min. |
| T3: Acetic acid for 40 min. | T4: Acetic acid for 60 min. |
| T5: Acetic acid for 120 min. | T6: Hot water for 20 min. |
| T7: Hot water for 40 min. | T8: Hot water for 60 min. |
| T9: Hot water for 120 min. | |
| TS1: Stratification + no treatment | |
| TS2 – TS5: acetic acid + stratification for the same periods. | |
| TS6 – TS9: Hot water + stratification for the same periods. | |

The treated and untreated seeds were planted at 2 cm depth in the plastic grow bags on October 2, 2016 and germination along with vegetative growth were monitored and recorded to May 2, 2017. Some weeding was done along with soil treatment with insecticides (TOPSIN-M and tachigazole) The following variables were investigated:

Germination percentage, plant height, leaf area, chlorophyll content, root length, fresh weight of the plant (leaves, shoots, and roots), fresh weight of roots, dry weight of leaves and shoots, dry weight of roots, and nitrogen, phosphorous, and potassium percentage in the dry plant tissues.

The results of the experiment were analyzed based on Randomized Complete Block Design (RCBD) with two factors and nine treatments (2 X 3 X 9), and each treatment with three replicates. Each experimental unit with five seeds. The comparison of the medians was done using Duncan's multivariates test at 0.05 and 0.01 probability.

RESULTS AND DISCUSSION

Seed Germination percentage:

Seed germination percentage was not significant for stratification, (Table 1). There are significant differences between treatments on germination; the highest germination percentage was obtained with hot water at 60 min. (T8) as it reached 96.67%, then T9, which did not significantly different as it got 93.33%. Other treatments T7-T1 are different as the germination percentages ranged between 13.33 – 80.00%. The combination of hot water treatment at 60 min. and no stratification gave 100.00% germination compared to 93.33% with stratification.

Seedling average height (cm):

The seedling average height was not significant with stratification but it was significant with treatments as appeared in Table (2). Treatment T9 (hot water for 120 min.), gave the highest seedling as they reached 7.41 cm compared to other treatments T8 – T1 as they got a range of 1.83 – 7.36 cm. The combination of stratification and acetic acid or hot water treatments was significant as T9S2 gave the highest seedlings reached 7.66 cm compared to T1S1, which got 1.00 cm of seedling height.

Table (1): Effect of stratification and no stratification with acetic acid and hot water on germination percentages.

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	13.33 b	33.33 b	23.33 c
T2	13.33 b	33.33 b	23.33 c
T3	20.00 b	6.67 c	13.33 d
T4	40.00 b	13.33 b	26.67 c
T5	13.33 b	40.00 b	26.67 c
T6	93.33 a	40.00 b	66.67 b
T7	73.33 a b	86.67 a	80.00 a b
T8	100.00 a	93.33 a	96.67 a
T9	93.33 a	93.33 a	93.33 a
Stratification average	51.11 a	48.88 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Table (2): Effect of stratification and no stratification with acetic acid and hot water on seedling average heights (cm).

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	1.00 d	2.68 cd	1.83 c
T2	4.00 a b c d	3.83 a b c d	3.92 b c
T3	3.44 a b c d	1.68 d	2.56 b c
T4	6.10 a b c	3.33 b c d	4.72 a b
T5	3.38 a b c d	4.21 a b c d	3.79 b c
T6	4.98 a b c d	4.94 a b c d	4.96 a b
T7	7.61 a b	7.11 a b	7.36 a
T8	6.69 a b c	7.28 a b	6.99 a
T9	7.16 a b	7.66 a	6.99 a
Stratification average	4.92 a	4.74 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Total chlorophyll contents (SPAD):

The treatments effect on total chlorophyll content results are in Table (3). The results of stratification did not reflect any significant differences, while the treatments clearly showed significant differences on chlorophyll content. Treatment T8 (hot water at 60 min.) obtained the highest value of chlorophyll as 37.43 SPAD compared to other hot water treatments (T9 and T7, they resulted in 34.76 and 33.26 SPAD respectively). There is a significant difference with the acetic acid treatments T2, T3, and the control treatments T1, in which the results were 24.11, 15.32, and 15.50 SPAD respectively. The combination of stratification and hot water and acetic acid treatments were significant as T8S1 gave the highest chlorophyll content of 37.36 SPAD compared to T1S1 which obtained only 10.00 SPAD.

Table (3): Effect of stratification, hot water, acetic acid, and their combinations on chlorophyll contents (SPAD).

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	10.00 c	21.00 a b c	15.50 b
T2	19.50 a b c	28.53 a b c	24.11 a b
T3	19.64 a b c	11.00 a b c	15.32 b
T4	31.50 a b c	18.33 a b c	24.92 a b
T5	28.80 a b c	23.80 a b c	22.30 a b
T6	31.69 a b c	31.82 a b c	31.75 a b
T7	32.98 a b c	36.53 a	34.76 a
T8	37.63 a	37.23 a	37.43 a
T9	31.79 a b c	34.73 a b	33.26 a
Stratification average	26.17 a	26.98 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Leaf area average (cm²):

The results in Table (4) showed that the effect of stratification, acetic acid, and hot water treatments are not significant when it comes to stratification while the treatments are significant. Treatment of hot water at 60 min. (T8) obtained the highest leaf area (12.86 cm²), compared with treatments T7 and T6 as they differed significantly from T9 (10.45 and 8.08 cm²). Treatments T5 – T1 also showed clear significant differences as the results ranged from 6.07 – 1.15 cm². The combinations of stratification and hot water or acetic acid did not give any differences as appeared in the table, 6.80 and 6.65 cm².

Table (4): Effect of stratification, hot water, acetic acid treatments and their combination with stratification on leaf area average (cm²).

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	0.29 d	2.00 c d	1.15 f
T2	2.41 c d	2.88 c d	2.65 e f
T3	2.80 c d	0.80 d	1.85 e f
T4	7.16 a b c	4.99 b c d	6.07 c d
T5	5.36 b c d	5.11 b c d	5.23 c d e
T6	9.00 a b	7.16 a b c	8.08 b c
T7	9.55 a b	11.39 a	10.45 a b
T8	13.17 a	12.55 a	12.86 a
T9	11.69 a	12.72 a	12.20 a
Stratification average	6.80 a	6.65 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Root length (cm):

Table (5), showed the results of stratification, acetic acid, and hot water treatments and their combinations effect on seedling root length. Stratification did not affect the root length significantly. While the treatments were significantly affected the root length. Treatment of hot water at 60 min. (T8), also got the highest root length (35.05 cm), followed by T9 which obtained (26.05 cm) root length, which is significantly different from treatments T7 – T1 as they got 23.14 – 4.69 cm. The combinations of stratifications and acetic acid or hot water, showed that T8S1 reached the highest root length as 43.47 cm compared to T1S1, which got 2.33 cm.

Table (5): Effect of stratification, acetic acid, hot water, and their combinations on root length (cm).

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	2.33 e	7.32 c d e	4.69 d
T2	7.03 c d e	27.27 a b	17.29 b c
T3	22.13 b c d	4.33 d e	13.23 d c
T4	20.17 b c d e	14.03 b c d e	17.10 b c
T5	10.33 b c d e	15.70 b c d e	13.02 d c
T6	15.72 b c d e	20.13 b c d e	17.92 b c
T7	23.22 b c d	23.05 b c d	23.14 b c
T8	43.47 a	26.68 a b	35.07 a
T9	25.50 b c	26.60 a b	26.05 a b
Stratification average	18.91 a	18.31 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Dry weight of the roots (gm.):

Table (6), shows the results of the effect of stratification, acetic acid, hot water, and combination on the dry weight of roots. There is no significant difference of stratification but there is a significant difference between treatments. Hot water treatment for 60 min. obtained the highest result of the dry weight of the roots (0.14 gm.). The treatment of acetic acid addition was significantly different from hot water as treatments of T5 – T2 as they gave dry weight of roots (0.06 – 0.05 gm.). The combinations of stratification and acetic acid or hot water is clearly different as the treatment of hot water for 20 min. got the highest dry weight of roots (S2T6 with 0.18 gm.).

Table (6): Effect of stratification, acetic acid, hot water, and the combinations treatments on the dry weight of roots (gm.).

Treatment/stratification	No stratification S1	Stratification S2	Treatment average
T1	0.003 b	0.05 b	0.005 a
T2	0.53 b	0.06 a b	0.05 a b
T3	0.08 a b	0.01 b	0.05 a b
T4	0.13 a b	0.02 b	0.07 a b
T5	0.04 a b	0.09 a b	0.06 a b
T6	0.05 a b	0.18 a	0.12 a
T7	0.13 a b	0.10 a b	0.12 a
T8	0.16 a b	0.13 a b	0.14 a
T9	0.12 a b	0.13 a b	0.14 a
Stratification average	0.09 a	0.08 a	

The similar letters mean no significant for each factor and the combinations based on Duncan's multi-variances at 0.05 probability.

Other results including the fresh weight of vegetative parts of the plant, dry weight of leaves and shoots, nitrogen, phosphorus, and potassium contents all showed that stratification did not affect those variables but the treatments were clear affected the variables. It was clear that acetic acid treatments were different from hot water and even with their combinations with stratification, than with hot water treatments especially hot water at 60 min. or 120 min. as they got the highest results of almost all the variables.

As far as we know that all seeds when they passed natural or artificial dormancy due to different conditions or requirements, these seeds start germination and form new plants. Whenever you put the seed in wet environment and oxygen available, they will grow as they start absorb water and the breaking down of reserved food start conversion into usable metabolites and germination happen. Before the germination when the seed is dry, the seed is a dry object and the metabolisms are at the lowest range. The seed coat is tough and does not allow water or gases to go in and let germination takes over. All dry seeds pass through this stage. In germination, water gets into the seed through seed coat and start swelling, the metabolites in seed changed into soluble materials and hydrolytic reactions occur in addition to gases exchange, in order for the germination to start. If the seed coat is not permeable for water due to physiological or natural dormancy, water cannot go into the seed and no germination will occur. This phenomenon is normal in plant life cycle to preserve the species in life cycle or overcome some unfavorable environmental conditions. However, when the plant seeds exposed to physiological dormancy due to some growth regulators such as abscisic acid, which extends the physiological dormancy (Xiaodong et al. 2013), which confirmed occurrence of that dormancy in newly harvested seeds. Other researchers connected this kind of dormancy as related to communication between ABA and AB15, AB14, and ABB pulses, they also concluded that the metabolisms and environmental conditions such as temperature, metabolisms after ripening, and nitrogen compounds control or affect that communication, (Matakiadis et al. 2009).

The seed dormancy in plant family Fabaceae species (as carob is a member of this plant family), is very much due to the hard seed coat which does not allow water or gases to go in or being exchanged. However, in this case we need to treat these seeds to enhance germination when plant them for our purposes. Many chemical, physical, and mechanical methods applied to do so on dormant seeds. In this experiment, hot water treatment increased germination from 23.33% to 97.67% by treating the seeds in hot water for 60 min. The increased germination to 97.67% may be due to increase the permeability of seed coat to water in which the hot water softened the seed coat and allowed water absorption and solubilize the reserved food substances in cotyledons of the seed and germination occurred. This finding agreed with Longer and Degago, (1996), as the germination enhanced by water absorption and gases exchange in addition to activation of metabolites conversion into usable formulas for metabolism. The gases exchange and water absorption could release the inhibitors in some of the seed embryo parts as phenolic compounds, (Mohammed –Yaseen et al., 1994), and

overcome the problem of seed coat hardness. This also, in agreement with what reported on hard seed coat of some other plants as Sumac tree, (Doassi and Thanos, 1994), Teak, (Yadav, 1992), many species of Acacia (NAS, 1980, Halle et al., 1981, and Bell and William, 1998).

The treatments of carob seeds with acetic acid as expected to improve the seed germination; it did but affected the vegetative growth of the seedlings and this is may be due to toxicity effect of the acetic acid on embryo parts as reported before on wheat by Tunes et al., (2012). Acetic acid is less toxicity but affected the treated seeds with acetic acid as reported by Lynch, (1980), on parley, wheat, carnation, corn, and grape seeds. As the addition of acetic acid controlled the fungal diseases acid and it did good at low concentrations, but the extension of acetic acid treatment time could damage the seed embryo parts in which the germination decreased as reported by Szopinska, (2013) on Zinnia ornamental plant (*Zinnia elegans* Jacq.).

CONCLUSIONS

In conclusion, hot water and acetic acid treatments with stratification were successful in increasing the seed germination of carob. Results from this experiment were similar, as the stratification did not influenced the germination when it was applied alone but it did with combination with hot water and acetic acid. The treatments with hot water and acetic acid for periods of time 20, 40, 60, and 120 min, in addition to the combinations with stratification increased the seed germination and vegetative growth parameters. The results that will be more applicable by farmers, ornamental nurseries, and forestry people are to use the hot water for 60 min. in which it approved more improvement of seed germination and affected vegetative growth parameters clearly.

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تطرية بذور الخروب بالماء الحار وحامض الخليك الثلجي والتنضيد البارد في الرمل لتحسين انبات البذور والنمو الخضري للشتلات النامية

خالد صبحي خليل ويونس حسين سليمان

قسم البستنة وهندسة الحدائق - كلية الزراعة - جامعة تكريت - العراق

المستخلص

اجريت هذه الدراسة في الظلة الخشبية والبيت الزجاجي\ قسم البستنة وهندسة الحدائق اكلية الزراعة اجامعة تكريت للمدة من 2016\10\2 الى 2017\5\2، لدراسة تأثير التنضيد البارد والمعاملة بالماء الحار وحامض الخليك في انبات بذور الخروب *Ceratonia siliqua* L. بالماء الحار لمدة 20 دقيقة و40 دقيقة و60 دقيقة و120 دقيقة ، والمعاملة بحامض الخليك لمدة 20 دقيقة و40 دقيقة و60 دقيقة و120 دقيقة إضافة الى معاملة المقارنة (بدون معاملة) والمعاملة بالتنضيد لمدة عشرة ايام على درجة حرارة 5م° والمعاملة بالماء الحار بعد التنضيد لمدة 20 دقيقة و40 دقيقة و60 دقيقة و120 دقيقة والنقع بحامض الخليك بعد التنضيد لمدة 20 دقيقة و40 دقيقة و60 دقيقة و120 دقيقة إضافة الى معاملة المقارنة (تنضيد فقط). تم تصميم التجربة حسب تصميم القطاعات العشوائية الكاملة مع ثلاثة مكررات وبواقع 10 بذرة لكل معاملة. بعد سبعة اشهر من زراعة البذور سجلت البيانات التالية : النسبة المئوية للإنبات ، ارتفاع الشتلات ، المساحة الورقية للنبات ، الكلوروفيل الكلي ، اطوال الجذور ، الوزن الرطب للمجموع الخضري ، الوزن الجاف للمجموع الخضري ، الوزن الرطب للمجموع الجذري ، الوزن الجاف للمجموع الجذري ، نسبة النتروجين ، نسبة الفسفور ،نسبة البوتاسيوم ، تشير النتائج الى الحصول على أفضل نسبة مئوية للإنبات 96.67% للمعاملة بالماء الحار لمدة 60 دقيقة بدون تنضيد، حصل على اعلى معدل للمساحة الورقية للنبات 12.86سم عند المعاملة بالماء الحار لمدة 60 دقيقة ،حصل اعلى معدل للوزن الرطب للمجموع الخضري 1.86 غم عند المعاملة بالماء الحار لمدة 40 دقيقة ، حصل اعلى معدل للوزن الجاف للمجموع الخضري 0.58 غم عند المعاملة بالماء الحار لمدة 60 دقيقة ، حصل اعلى معدل للوزن الرطب للمجموع الجذري 7.05 غم عند المعاملة بالماء الحار لمدة 40 دقيقة ، حصل على اعلى معدل للوزن الجاف للمجموع الجذري 0.14 غم عند المعاملة بالماء الحار لمدة 60 دقيقة ، حصل على اعلى معدل لنسبة النتروجين في الاوراق 5.52% عند المعاملة بالماء الحار لمدة 60 دقيقة ، حصل اعلى معدل لنسبة الفسفور في الاوراق 0.49% عند المعاملة بالماء الحار لمدة 120 دقيقة، حصل اعلى معدل لنسبة البوتاسيوم في الاوراق 2.07% عند المعاملة بالماء الحار لمدة 120 دقيقة .

الكلمات المفتاحية: تطرية، خروب، التنضيد، الماء الحار، حامض الخليك الثلجي، انبات البذور.