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**RESEARCH ARTICLE**

# Germination of Pomegrate Seeds *(Punica granatum L.)* on Concentration Treatment and Soaking Time in Sulfuric Acid ($H_2SO_4$)

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**ABSTRACT**

One of the medicinal plants that contain high antioxidants and have an important role in nutritional needs is pomegranate. Pomegranate plant propagation can be done generative and vegetatively. Sexual propagation is constrained because pomegranate plants have dormancy properties and hard seed coats that cause seed germination to take a long time. Effectiveness of sulfuric acid is used to break dormancy in hard seed coats so that the inhibition process goes well, and the germination process is faster. This research aims to determine the effect of concentration and duration of immersion in sulfuric acid solution on the germination of pomegranate seeds. This research used a two-factor randomized block design. The first factor was sulfuric acid concentration (control, 65%, 70%, 75%). The second factor is the immersion time (without immersion, 5 minutes. 10 minutes. 15 minutes). Each treatment was repeated 3 times.



## 1. INTRODUCTION

Pomegranate is a plant that originates from Central Asia such as Iran, Afghanistan, and the Himalayan Mountains. Pomegranate is one of the oldest fruits that has an important role in nutritional security, such as supplements, food, and medicine. Pomegranates are often planted in home gardens as ornamental plants and the fruit is used for food. Pomegranate contains tannins which can protect the heart. Apart from containing antioxidants, pomegranate also contains provitamin A, vitamin C, vitamin B1, vitamin B2, iron, potassium, polyphenols (3 times more than green tea), and flavonoids (Ide, 2012).

Pomegranate plant propagation can be done generatively and vegetatively. The problem with the generative propagation of pomegranates is that the seeds have dormancy properties where the seed coat is hard (Candra et al., 2017; Kumar, 2014). The hardness of the seed coat can cause pomegranate seeds to germinate for a very long time (Materechera & Seeiso, 2011).
Imbibition is the entry of water into the seed so that the water content in the seed reaches a certain percentage. The process of breaking dormancy in thick and hard-skinned seeds is carried out by soaking the seeds in chemical solutions such as sulfuric acid, nitric acid, potassium hydroxide, hydrochloric acid, potassium nitrate, and thiourea (Sutopo, 2010). Soaking seeds in a concentrated solution of $H_2SO_4$ and HCl makes the seed coat softer so that water can pass through easily (Fahmi, 2012).

Breaking dormancy in pomegranate seeds chemically with different concentrations produces different results (Gokturk et al., 2022; Olmez, 2007). Treatment with 70% $H_2SO_4$ for 15 minutes produced a normal germination percentage, which is 90% with a germination rate of 14.04 days (Ramadhani, 2014). This research was conducted to determine the effect of the concentration of sulfuric acid ($H_2SO_4$) and the length of soaking time on pomegranate seeds to increase pomegranate seed germination.

## 2. MATERIALS AND METHODS

This research was conducted in Hutapadang Village, Kotanopan District, Mandailing Natal Regency, North Sumatra, and Agroecotechnology Laboratory, Faculty of Agriculture, Universitas Malikussaleh. The research was done from February to March 2021.

The tools used in this research were sprout tubs, beakers, measuring cups, measuring flasks, hand sprayers, knives, cameras, stationery, sieves, label paper, and other equipment that supported this research. The materials used are Indian red pomegranate seeds, topsoil, river sand, water, distilled water, and sulfuric acid solution ($H_2SO_4$).

This research used a factorial Randomized Block Design (RBD) with two treatment factors, which are the concentration of the $H_2SO_4$ solution (K) and the length of soaking time (P). Factor I: The concentration of the $H_2SO_4$ (K) solution consists of 4 levels, which are: K0 (Control), K1 (with 65% of $H_2SO_4$), K2 (with 70% of $H_2SO_4$), K3 (with 75% of $H_2SO_4$). Factor II: The length of soaking the seeds in the $H_2SO_4$ (P) solution consists of 4 levels, which are: P0 (No Soaking), P1 (5 minutes of Soaking), P2 (10 minutes of Soaking), P3 (15 minutes of Soaking). Thus, there were 16 treatment combinations with 3 repetitions so there were 48 experimental units.

The variables observed in this research were maximum growth potential (%), germination capacity (%), growth simultaneity (%), growth speed (%), and vigor index (%). Data analysis was carried out using ANOVA. If the results of the analysis show different results, it will be continued with Duncan's Multiple Range Test (DMRT) at the 0.05 level.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Results

#### 3.1.1. Maximum Growth Potential, Germination Capacity, Growth Simultaneity, Growth Speed, and Vigor Index

The concentration of the $H_2SO_4$solution and the length of soaking alone showed a significant influence on the variables of maximum growth potential, germination capacity, growth simultaneity, growth speed, and vigor index. Further test results from the treatment of $H_2SO_4$ solution concentration and soaking time on variables of maximum growth potential, germination capacity, growth simultaneity, growth speed, and pomegranate seed vigor index are presented in Table 1.

Table 1. Effect of Sulfuric Acid Concentration ($H_2SO_4$) and Length of Soaking on Maximum Growth Potential, Germination Capacity, Growth Simultaneity, Growth Speed, and Vigor Index of Pomegranate Seeds

| Treatment | Maximum Growth Potential (%) | Germination Capacity (%) | Growth Simultaneity (%) | Growth Speed (%) | Vigor Index (%) |
|---|---|---|---|---|---|
| Concentration of $H_2SO_4$ (K) | | | | | |
| K0 (0 % of $H_2SO_4$) | 42.67 c | 32.33 c | 16.00 c | 10.99 d | 6.33 d |
| K1 (65% of $H_2SO_4$ ) | 57.00 b | 39.67 b | 27.67 b | 17.63 c | 11.67 c |
| K2 (70% of $H_2SO_4$) | 57.33 b | 45.33 a | 34.67 a | 23.57 b | 15.67 b |
| K3 (75% of $H_2SO_4$) | 63.00 a | 47.00 a | 35.00 a | 25.97 a | 19.67 a |
| Length of Soaking $H_2SO_4$ (P) | | | | | |
| P0 (Without Soaking) | 48.67 b | 38.67 c | 25.33 b | 17.46 c | 11.33 c |
| P1 (5 minutes of Soaking) | 52.33 b | 33.67 d | 24.33 b | 16.88 c | 11.67 c |
| P2 (10 minutes of Soaking) | 58.33 a | 43.00 b | 31.67 a | 20.76 b | 14.00 b |
| P3 (15 minutes of Soaking) | 60.67 a | 49.00 a | 32.00 a | 23.06 a | 16.33 a |

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT test 5%.

Table 1 shows that the concentration of $H_2SO_4$ solution can increase the maximum growth potential, germination capacity, growth simultaneity, growth speed, and vigor index of pomegranate seeds. The best treatment is K3 (75% of $H_2SO_4$). The length of soaking also influences the maximum growth potential, germination capacity, growth simultaneity, growth speed, and vigor index of pomegranate seeds. The best treatment is P3 treatment (15 minutes soaking).

The interaction between the concentration of the $H_2SO_4$ solution and the length of soaking can be seen in the variables of maximum growth potential, germination capacity, growth simultaneity, growth speed, and vigor index. Further test data on the interaction between the concentration of the $H_2SO_4$ solution and the length of soaking are presented in Table 2. The results of the research show that the interaction of the two factors can increase the maximum growth potential, germination capacity, simultaneous growth, growth speed, and vigor index of pomegranate seeds.

Table 2. The Interaction of Sulfuric Acid Concentration ($H_2SO_4$) and Length of Soaking on Maximum Growth Potential, Germination Power, Simultaneity of Growth, Growth Speed, and Vigor Index of Pomegranate Seeds

| Treatment | | Maximum Growth Potential (%) | Germination Capacity (%) | Growth Simultan (%) | Growth Speed (%) | Indeks Vigor (%) |
|---|---|---|---|---|---|---|
| Concentration of $H_2SO_4$ (%) | Length of Soaking $H_2SO_4$ (minutes) | | | | | |
| 0 | 0 | 36,00 h | 32,00 fgh | 16,00 e | 9,22 fg | 4,00 i |
| 0 | 5 | 38.67gh | 25.33 h | 12.00 e | 8.66 g | 5.33 hi |
| 0 | 10 | 52.00 def | 44.00 d | 17.33 e | 14.65 e | 9.33 fg |
| 0 | 15 | 44.00 fg | 28.00 gh | 18.57 ed | 11.46 efg | 6.67 ghi |
| 65 | 0 | 62.67 b | 52.00 bc | 38.67 b | 25.84 c | 17.33 c |
| 65 | 5 | 46.47 efg | 28.00 gh | 17.33 c | 10.99 efg | 6.67 ghi |
| 65 | 10 | 60.00 bcd | 45.33 cd | 28.33 c | 19.41 d | 13.33 de |
| 65 | 15 | 58.67 bcd | 33.33 fg | 25.33 cd | 14.27 e | 9.33 fg |
| 70 | 0 | 49.33 ef | 37.33 ef | 29.33 c | 21.90 d | 16.00 dc |
| 70 | 5 | 65.33 b | 48.00 bcd | 41.33 b | 27.85 c | 18.67 c |
| 70 | 10 | 61.33 bc | 54.67 b | 41.33 b | 26.09 c | 17.33 c |
| 70 | 15 | 53.33 cde | 41.33 de | 28.00 c | 18.44 d | 10.67 ef |
| 75 | 0 | 46.67 efg | 33.33 fg | 17.33 e | 12.86 ef | 8.00 fgh |
| 75 | 5 | 58.67 bcd | 33.33 fg | 26.67 c | 20.05 d | 16.00 cd |
| 75 | 10 | 60.00 bcd | 52.00 bc | 38.67 b | 32.09 b | 25.33 b |
| 75 | 15 | 86.67 a | 69.33 a | 56.00 a | 38.87 a | 29.33 a |

Note: Numbers followed by the same letter in the same column are not significantly different according to DMRT test 5%.

## 3.2 Discussions

Based on observation data. it is known that the concentration of H2SO4 has a significant effect on maximum growth potential. The length of soaking has a significant effect on maximum growth potential. as well as the combination of the two treatments which has a significant effect on this variable. The maximum growth potential for the H2SO4 concentration and length of soaking is presented in Table 1 where the highest growth potential was found in the K3 treatment (63%) and was significantly different from the K0. K1. and K2 treatments. In the length soaking treatment. the highest growth potential was found in the P3 treatment (60.67%) and was not significantly different from the P2 treatment. but was significantly different from the P0 and P1 treatments. The combination of H2SO4 concentration and length of soaking is presented in Table 2 where the highest growth potential was found in the K3P3 treatment (86.67%) and was significantly different from the other treatments. The higher the H2SO4 concentration. the higher the average percentage of maximum growth potential will be. This is due to the high concentration of sulfuric acid. which makes the seed coat soft and causes the seed's ability to absorb water to become higher (Shalimu at al. 2015).

Based on observation data. it is known that the concentration of H2SO4 has a significant effect on germination capacity. The length of soaking and combination treatment has a significant effect on germination capacity. Germination capacity in the combination of H2SO4 concentration and length of soaking is presented in Table 1 where the highest germination capacity was found in the K3 treatment (47%) which was not significantly different from the K2 treatment but was significantly different from the K0 and K1 treatments. In the length of soaking treatment. the highest germination capacity was found in the P3 treatment (49%) which was significantly different from the P0. P1. and P2 treatments.

The combination of H2SO4 concentration and soaking time is presented in Table 2 where the highest germination capacity was found in the K3P3 treatment (69.33%) which was significantly different from the other treatments. Seed germination is the initial stage of a plant's development. The high percentage of germination capacity was found in the treatment of 75% of H2SO4 for 15 minutes (K3P3) which is thought to be due to the structure of the seed coat being damaged so that water can easily enter and the embryo can come out and germinate.

Based on observation data. it is known that $H_2SO_4$ concentration has a significant effect on growth simultaneity. The length of soaking and the combination treatment had a significant effect on growth simultaneity. Growth simultaneity on $H_2SO_4$ concentration and length of soaking is presented in Table 1 where the highest growth simultaneity was in the K3 treatment (35%) which was not significantly different from the K2 treatment. but significantly different from the K0 and K1 treatments. In the length soaking treatment. the highest growth simultaneity was in the P3 treatment (32%) which was not significantly different from the P2 treatment. but was significantly different from the P0 and

P1 treatments. The combination of $H_2SO_4$ concentration and length of soaking is presented in Table 2 where the highest growth simultaneity was found in the K3P3 treatment (56%). which was significantly different from the other treatments. The increase in growth simultaneity is thought to be due to soaking the seeds in sulfuric acid. so water absorption by the seeds (imbibition process) increases because the seed coat becomes softer. This shows that 75% sulfuric acid ($H_2SO_4$) treatment for 15 minutes (K3P3) can improve seed vigor both through growth speed and growth simultaneity.

Based on observation data. it is known that the concentration of $H_2SO_4$ has a significant effect on growth speed. The length of soaking and combination treatment had a significant effect on growth speed. The growth speed at different $H_2SO_4$ concentrations and soaking times is presented in Table 1 where the highest growth speed was found in the K3 treatment (25.97%) which was significantly different from the K0. K1. and K2 treatments. In the length of soaking treatment. the highest growth speed was in treatment P3 (23.06%) which was significantly different from treatments P0. P1. and P2. The combination of $H_2SO_4$ concentration and length of soaking is presented in Table 2 where the highest growth speed was found in the K3P3 treatment (38.87%) which was significantly different from the other treatments. Soaking seeds with $H_2SO_4$ is more optimal and faster for softening the seed coat than those that are only soaked in water for the same soaking time. Soaking seeds with a concentration of 75% for 15 minutes is effective for measuring the growth speed and is one of the vigor of growing strength. The high difference in vigor index and control treatment indicated that it was not effective enough for seed growth. A high vigor index indicates that the speed of seed germination is also high and they are more resistant to unfavorable environmental conditions (Satya et al.. 2015; Da Silva et al.. 2017).

Based on observation data. it is known that the concentration of $H_2SO_4$ has a significant effect on the vigor index. The length of soaking and the combination of treatments had a significant effect on the vigor index. The vigor index at different $H_2SO_4$ concentrations and soaking times is presented in Table 1 where the highest vigor index was found in the K3 treatment (19.67%) which was significantly different from the K0. K1. and K2 treatments. In the length of soaking treatment. the highest vigor index was found in the P3 treatment (16.33%) which was significantly different from the P0. P1. and P2 treatments. The combination of the $H_2SO_4$ concentration treatment and length of soaking is presented in Table 2 where the highest vigor index was found in the K3P3 treatment (29.33%) which was significantly different from the other treatments.

## 4. CONCLUSION

The germination value of red pomegranate seeds increases due to 75% $H_2SO_4$ and 15 minutes of soaking.

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