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Employing HCl for Pulp Extraction and Its Effect on Viability and Vigor of Cocoa Seeds (*Theobroma cacao* L.) Variety TSH 858

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Abstract
Cocoa seeds are classified as recalcitrant and have a pulp layer containing corrosive substances such as abscisic acid; if not properly cleaned, this layer can inhibit the germination process. To date, manual pulp cleaning using scouring ash remains ineffective and time-consuming. An alternative method is chemical treatment, such as using hydrochloric acid (HCl). The application of HCl at specific concentrations has been shown to effectively dissolve and break down the pulp layer attached to the seeds without damaging the seed tissue. This study aims to evaluate the effectiveness of various HCl concentrations for pulp extraction and their impact on the viability and vigor of cocoa seeds of the TSH 858 variety. The research was conducted at the Seed Technology Laboratory of the Faculty of Agriculture, Andalas University, employing a Completely Randomized Design (CRD) with five treatments: 0% HCl (control), 1% HCl, 3% HCl, 5% HCl, and 7% HCl. Each treatment was replicated four times. Data were analyzed using the F-test at a 5% significance level, followed by Duncan's New Multiple Range Test (DNMRT) at the same level if a significant effect was detected. The parameters measured included seed moisture content, germination rate, seedling height, root length, and vigor index. The results indicated that treatment with 5% HCl yielded the best outcomes for seed vigor, particularly in enhancing root length, but had no significant effect on seed viability. Therefore, 5% HCl is effective for extracting cocoa seed pulp without compromising viability and can improve seed vigour by promoting root growth.

Keywords: Chemical Substances, Concentration, Hydrochloric Acid, Recalcitrant, Vigor Index

1. Introduction

Cocoa (*Theobroma cacao* L.) is a plantation crop that plays a vital role in the national economy. Compared to other countries such as Ghana, Indonesia's cocoa productivity is only half as high. In fact, Indonesian cocoa production declined by 5.46% in recent years (2021–2022) (BPS-Statistics Indonesia, 2023). This decline will undoubtedly affect the country's export value and have a direct impact on the income of cocoa farmers. One way to address this issue is through upstream activities, particularly by providing high-quality, superior seeds in the right quantities and at the right time.

Cocoa seeds are recalcitrant (Baharudin et al., 2010), and their pulp contains corrosive abscisic acid (Valentina et al., 2020), which can inhibit germination if not properly removed. Manual pulp cleaning can be performed using scouring powder; however, this method remains ineffective and time-consuming on a large scale..

The pulp attached to cocoa beans will slow seed germination and cause microbiological contamination if not removed (Bahri et al., 2021). Cocoa pulp can be removed using several methods, including mechanical, manual, and chemical processes. Mechanical pulp removal or extraction carries a high risk of seed damage, whereas manual pulp removal is less efficient due to its reliance on human labour, which can affect the results and require a considerable amount of time (Abdullah, 2022).

The use of chemicals is an alternative option that can be applied because it can completely remove the pulp from cocoa seeds in a short time. Some substances that can be used for pulp extraction include sulfuric acid (H2SO4), sodium hypochlorite (NaOCl), potassium nitrate (KNO3 and hydrochloric acid (HCl) (Tarigan & Mariati, 2018). Hydrochloric acid (HCl) is one of the chemicals that can dissolve and break down the pulp layer attached to the seed surface, and it is more affordable than other acid solutions

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(Widiarti et al., 2016).

Soaking mangosteen seeds in a 7% HCl solution for 30 minutes not only facilitates pulp extraction but also accelerates the germination rate by 2 days, with the percentage of normal germination reaching 70%, compared to seeds not soaked in HCl (Tarigan & Mariati, 2018). Similarly, studies on coffee seeds have shown that soaking in a 3% HCl solution can enhance germination vigor and promote early seedling growth (Junita et al., 2023). However, the use of HCl for pulp extraction in cocoa seeds remains uncommon. Therefore, this study aims to evaluate the effectiveness of various HCl concentrations for pulp extraction and their impact on the viability and vigor of cocoa seeds from the TSH 858 variety.

2. Material and Methods

The research was conducted from May to July 2025 at the Seed Technology Laboratory, Faculty of Agriculture, Andalas University, Padang. The materials and tools used in the research included cocoa pods of the TSH 858 variety, 32% HCl, distilled water, stencil paper, label paper, fungicide, seedbed, soil, sand, knife, hand sprayer, personal protective equipment, office stationery, digital scales, dropper pipettes, measuring cups, and several other supporting tools.

This research is the initial stage in preparing cocoa plant seeds that, in the long term, can be used to provide high-quality cocoa seeds for the Faculty of Agriculture at Andalas University. The research was conducted using a Completely Randomized Design (CRD) consisting of five treatments: 0% HCl, 1% HCl, 3% HCl, 5% HCl, and 7% HCl. Each treatment was repeated four times, with each experimental unit using 25 seeds. Data were analyzed using the F-test at a 5% level, and further analysis continued with the DNMRT test at a 5% level if a significant effect was detected. Data analysis was conducted using the STAR (Statistical Tool for Agricultural Research) software, version 2.0.1.

The stages of research activities began with a survey of superior cocoa plants, the selection of cocoa fruit, sterilization of planting media, preparation of test seeds, dilution of HCl solution, treatment, planting seeds in germination media, maintenance, and observation of seed viability and vigour (Figure 1). Cocoa fruit of the TSH 858 variety was obtained from the Cocoa Seed Garden of CV. Scorpio Communications is located in Luak Mayan, Jorong Gando, Piohang, Payakumbuh District, Lima Puluh Kota Regency. The cocoa fruit used met the criteria of being physiologically ripe, healthy, reddish-yellow in colour, and free from pests and diseases. Test seeds were obtained from the cocoa fruit by cutting the fruit horizontally with a knife. The seeds in the centre of the fruit were then selected and reselected to ensure uniformity of seed size.

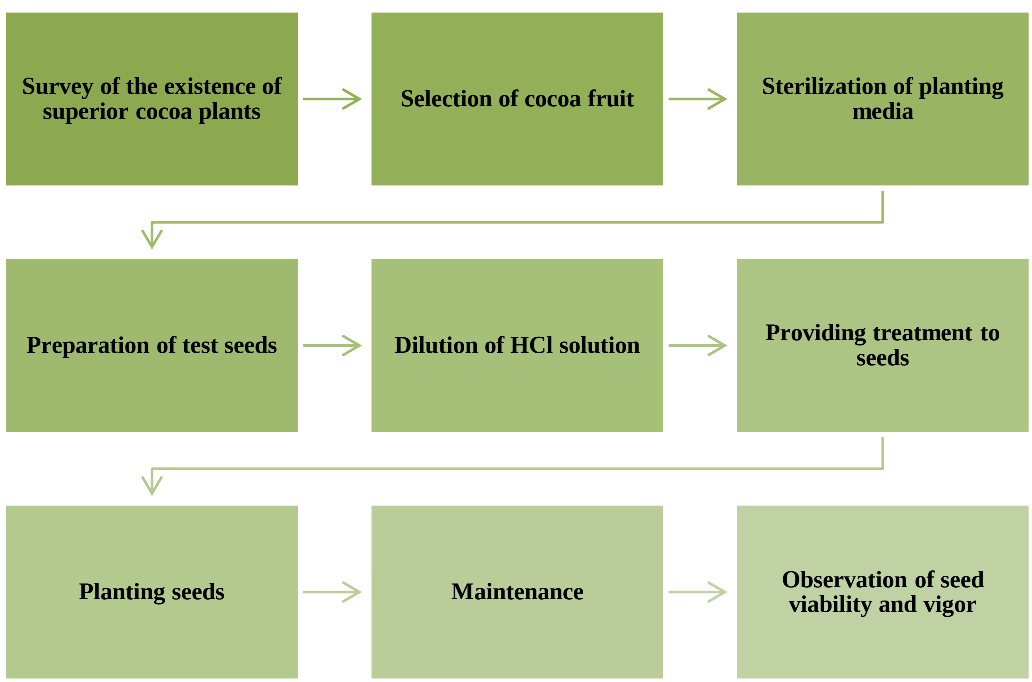

Figure 1. Research stages

The HCl solution used is 32% hydrochloric acid (HCl). The dilution formula for the solution used is as follows:

$M1 \times V1 = M2 \times V2$ ……. (Raviolo et al., 2021).

Information:

M1 = Initial concentration of HCl (32%)

V1 = Volume of HCl to be used

M2 = Desired HCl concentration (1%, 3%, 5% and 7%)

V2 = Volume of HCl to be made (1000 ml)

The prepared cocoa seeds were placed in a container.

For the control (0% HCl concentration), they were then soaked in distilled water for 30 minutes, kneaded, and filtered. Meanwhile, the seeds for the 1%, 3%, 5%, and 7% HCl treatments were each soaked in HCl according to the treatment for 30 minutes, kneaded, and then filtered; they were subsequently rinsed with running water. After seed treatment, to prevent mould, each treated seed was soaked in fungicide for 10 minutes. Then, the seeds were drained and air-dried for 1 hour on stencil paper.

The seeding medium, consisting of a 1:1 ratio of soil and sand, was sterilized using an autoclave for 15 minutes at 121°C. The sterile seeding medium was then placed in the seedbed. Observations were made on seed moisture content measurements using the oven method, seed germination power based on the International Rules for Seed Testing (ISTA), 2018), seedling height, root length, and vigor index based on the International Rules for Seed Testing (ISTA, 2018).

3. Results and Discussion

3.1. Seed moisture content (%)

Based on the analysis of variance of the water content in cocoa seeds of the TSH 858 variety after immersion in HCl solutions at various concentrations, the data presented in Table 1 indicate that the HCl treatment had no significant effect on the water content of the seeds.

Table 1. Water content of TSH 858 cocoa seeds after being given the HCl solution soaking treatment

HCl concentration (%)	Water content (%)
0	51.38 ± 4.78
1	50.84 ± 0.53
3	51.97 ± 2.94
5	49.34 ± 4.85
7	53.64 ± 2.45
KK	6.81%

Description: The numbers in the same column are not significantly different according to the F test at the 5% significance level.

The moisture content of cocoa seeds of the TSH 858 variety after HCl immersion treatment ranged from 49.34% to 53.64%. This value indicates that the cocoa seeds still have a high moisture content and are classified as recalcitrant seeds, which are naturally not resistant to drying and require relatively high moisture content to maintain seed viability (Berjak & Pammenter, 2008).

Treatment with various HCl concentrations (1–7%) did not significantly affect seed moisture content compared to the control. This finding is consistent with the primary function of HCl in seed treatment, which is to remove pulp from cocoa seeds, not to reduce the internal moisture content of the seeds. Interestingly, the seed moisture content in the 5% HCl treatment decreased slightly (49.34%), whereas in the 7% treatment, it increased (53.64%). This fluctuation is likely influenced by differences in water imbibition capacity among the various treatments (Gomez & Gomez, 2010).

3.2. Seed germination power (%)

Based on the analysis of variance on the germination power of cocoa seeds of the TSH 858 variety after immersion in HCl solution at several concentrations, the data obtained are presented in Table 2. The HCl solution immersion treatment had no significant effect on seed germination.

The use of HCl in cocoa seed treatment aims to remove the pulp or mucus adhering to the seed surface. The HCl concentration used is optimal, ensuring it does not damage the seed embryo and effectively eliminates the pulp layer. Cocoa seeds have been shown to have a germination rate ranging from 88% to 95%, exceeding the minimum optimal germination limit of 80% (International Rules for Seed Testing (ISTA), 2018).

The attached pulp can contain sugars and organic compounds that serve as a growth medium for microorganisms, potentially reducing seed viability during germination if not removed. Demucilaging cocoa seeds is a simple yet crucial treatment for maintaining viability and increasing vigor. Leaving the pulp attached accelerates seed deterioration due to microbial activity and high respiration. Therefore, demucilaging is highly recommended before planting to ensure the high physiological quality of cocoa seeds (Adu et al., 2017).

Table 2. Germination power of TSH 858 cocoa seeds after being given HCl solution soaking treatment

HCl concentration (%)	Normal sprouts (%)	Abnormal sprouts (%)	Dead sprouts (%)
0	95 ± 6.00	0 ± 0	5.00 ± 6.00
1	90 ± 8.33	0 ± 0	10.0 ± 8.33
3	94 ± 2.31	0 ± 0	6.00 ± 2.31
5	94 ± 7.66	0 ± 0	6.00 ± 7.66
7	88 ± 9.24	0 ± 0	12.0 ± 9.24
KK	7.74%	0%	9.15%

Description: The numbers in the same column are not significantly different according to the F test at the 5% significance level.

Soaking in HCl up to 7% was effective in cleaning the pulp of TSH 858 cocoa seeds without reducing germination or vigor, although high concentrations showed a tendency to increase seed mortality.

3.3. Sprout height (cm) and root length (cm)

Based on the analysis of variance on the height of the sprouts and the length of the roots of cocoa seeds of the

TSH 858 variety after being given the treatment of soaking in HCl solution at several concentrations, the data obtained were as presented in Table 3 where the treatment of soaking in HCl solution did not have a significant effect on the height of the sprouts but had a substantial impact on the length of the roots:

Table 3. Height of sprouts and length of roots of TSH 858 cocoa seeds after being given HCl solution soaking treatment.

HCl concentration (%)	Sprout height (cm)	Root length (cm)	
0	6.79 ± 1.08	8.63 ± 0.95	Bc
1	7.66 ± 3.78	9.75 ± 0.83	B
3	8.22 ± 1.73	8.35 ± 0.99	C
5	8.21 ± 1.64	12.28 ± 0.61	A
7	6.04 ± 1.95	7.33 ± 0.71	C
KK	30.26%	8.99%	

Description: The numbers in the same column followed by different lowercase letters are significantly different according to the DNMRT test at the 5% level, but the numbers in the column not followed by lowercase letters are not significantly different according to the F test at the 5% level.

The average seedling height ranged from 6.04 to 8.22 cm, with the highest values in the 3% and 5% HCl treatments (±8.2 cm), and the lowest in the 7% treatment (6.04 cm). Although there was variation, this difference was not statistically significant. This finding indicates that the pulp cleaning treatment using HCl did not affect the growth of the epicotyl. This condition may be attributed to the relatively high viability of cocoa seeds across all treatments, leading to normal initial seedling growth (Seno *et al.* 2023).

HCl treatment significantly affected root length. The highest value was obtained at a concentration of 5% (12.28 cm), while the lowest was at a concentration of 7% (7.33 cm). This finding indicates that a certain (optimal) HCl concentration can enhance root growth, while higher concentrations can cause toxic effects. Longer root growth at a concentration of 5% HCl indicates optimal conditions that support the process of imbibition and root respiration. However, at a concentration of 7%, the HCl solution may be too strong, causing osmotic stress or damage to root tissue. The use of chemicals for seed cleaning can improve root growth, but excessive doses have the potential to damage root meristem tissue (Feng et al., 2022; Jabbour & Alzahrani, 2024; Orzali et al., 2023; Yuan & Huang, 2016).

3.4. Vigor index (%)

Based on the analysis of variance of the vigour index of cocoa seeds of the TSH 858 variety after immersion in HCl solution at several concentrations, the data obtained are presented in Table 4. The HCl solution immersion treatment did not have a significant effect on the vigour index.

The cocoa seed vigor index ranged from 81% to 89%, with the highest value observed in the 5% HCl treatment (89%) and the lowest in the 7% HCl treatment (81%). The consistently high vigor index values across all treatments (>80%) indicate that soaking in HCl to remove pulp did not compromise the physiological quality of the seeds. Although the differences were not statistically significant, the highest vigor index in the 5% HCl treatment aligns with the root length results presented in Table 3, which were also optimal at this concentration. These findings suggest that moderate HCl concentrations effectively remove pulp without inducing chemical stress in the seeds. In contrast, higher acid concentrations can reduce the seed vigor index (Tanjung et al., 2017).

Table 4. Vigor index of TSH 858 cocoa seeds after being given HCl solution soaking treatment

HCl concentration (%)	Vigor index (%)
0	88 ± 3.27
1	84 ± 7.30
3	88 ± 3.27
5	89 ± 6.38
7	81 ± 8.25
KK	7.16%

Description: The numbers in the same column are not significantly different according to the F test at the 5% significance level.

4. Conclusion

The application of 5% HCl yielded the best results for seed vigor, particularly in enhancing root length, but had no significant effect on seed viability. Therefore, 5% HCl is effective for extracting cocoa seed pulp without compromising viability and can increase seed vigor by promoting root growth.

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