The effect of seeds soaking duration in alcohol of *In vitro* germination *Sesbania grandiflora*

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Abstract. Turi (Sesbania grandiflora) is a nutrient-rich legume. All parts of the turi plant can be used as a source of animal feed. Turi plants have hard seeds, which is a limitation in cultivation and are relatively slow to regrow after being cut. This study aims to determine the sterilization concentration of turi seeds using alcohol to accelerate the in vitro germination. This research uses an experimental method consisting of 6 treatments, namely P0: no alcohol soaking, P1: soaked in 70% alcohol for 5 minutes, P2: 10 minutes, P3: 15 minutes, P4: 20 minutes, and P5: 25 minutes. The variables measured are initial germination time (days), germination rate (%), and contamination percentage (%). The results of the study showed that the average germination time ranged from 0 to 13.00 days, the average germination rate ranged from 0 to 46.00%, and the contamination percentage ranged from 0% to 45%. The diversity analysis results on the treatment of soaking turi seeds in alcohol regarding germination time, germination rate, and contamination percentage could not be statistically tested because the data did not meet the requirements for representative data for statistical analysis, as less than 50% of the seeds germinated.

1 Introduction

The conventional cultivation of fodder crops is a common method used to increase the availability of green fodder. However, for plants that are increasingly difficult to find, and whose seeds are hard and have low seedling growth due to slow growth after being cut, an appropriate method is needed to overcome these issues in order to improve their availability, so that the plants always exist and do not become extinct.

Turi (*Sesbania grandiflora*) is a plant that has hard seeds. Planting turi using seeds requires scarification because turi seeds have hard seed coats, resulting in physical dormancy [1]. This physical dormancy causes structural constraints in the germination process. Besides the hardness of the seeds, another limitation for regenerating turi plants is the difficulty in growth and elongation of trunks that have been cut. Red turi plants are slow to regrow when cut [2].

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On the other hand, this plant has many benefits; it can be used as a medicinal plant, an ornamental plant, and a source of concentrated feed for livestock. Because of the numerous uses of turi, efforts are needed to improve genetic quality through genetic engineering. One method is through tissue culture using the in vitro technique. The challenge in utilizing the in vitro method is achieving sterilization, and for seed sterilization, one of the commonly used substances is alcohol. Alcohol not only acts as a chemical to break physical dormancy in seeds but also serves as a sterilizing agent. In the context of in vitro seed planting techniques, the use of alcohol can enhance sterilization. Seventy percent alcohol is an effective and practical sterilizing material in plant tissue culture, especially as an initial step in seed sterilization. Choosing the appropriate soaking duration is crucial for success in in vitro culture.

Previous research on the use of 70% alcohol as a seed sterilization agent for turi plant seeds in tissue culture without soaking has successfully induced shoots of turi plants (*Sesbania grandiflora*) in vitro [3] and induced roots. However, the study of seed sterilization has not yet examined the appropriate concentration of alcohol and the soaking duration for turi plant seeds and too the addition of growth hormones in the germination medium. The success of a tissue culture, besides depending on the nutrients and chemical compounds contained in a type of medium, also heavily depends on the growth hormones added to the medium, because growth hormones play an important role in the growth and development of the culture [4].

2 Materials and Methods

The study was conducted using a Completely Randomized Design (CRD) consisting of 6 treatments and 5 replications, resulting in a total of 30 experimental units. Observations were made descriptively. Each culture bottle contained 5 explants. Hormone treatment was applied by adding 2.5 ml of *Butiric Acid Purin* (BAP) to each treatment. The treatments given involved seed sterilization using alcohol with different soaking times as follows: P0 = 70% Alcohol Soaking (control); P1 = Soaked in 70% alcohol for 5 minutes; P2 = Soaked in 70% alcohol for 10 minutes; P3 = Soaked in 70% alcohol for 15 minutes; P4 = Soaked in 70% alcohol for 20 minutes; P5 = Soaked in 70% alcohol for 25 minutes. The parameters observed were the initial germination time (days), germination rate (%), and contamination percentage (%).

Observations were made by looking at the initial germination time, which was measured by observing the first time the seeds sprouts, Germination rate is the number of normal seedlings produced within a specified period, The percentage of contamination is observed from daily seeds observations, and the sources of contamination from both bacteria and fungi are examined.

2.1 Workplace and Equipment Sterilization

Sterilization of the work environment and equipment used in the laboratory [5] includes: maintaining the cleanliness of all rooms used, such as the culture room, planting room, and stock room. The Laminar Air Flow is sterilized using UV light for 30 minutes, and the surface of the Laminar Air Flow Cabinet (LAFC) is sprayed with 70% alcohol.

2.2 Preparation of Germination Media

The germination medium used was MS (*Murashige and Skoog*) enriched with 2.5 ppm BAP hormone for each germination medium. The activities were carried out in the laboratory [6]. The germination medium used was MS with the addition of 2.5 ppm BAP hormone, prepared in a total of 1 liter. After mixing, the pH was measured using pH paper, with the expected pH being 5.8-6. Then, 7.5 g/L of agar was added as a solidifying agent, and the medium was heated and stirred using a hot plate magnetic stirrer until it boiled and the color was clear. The medium was then stored in the culture room for 1 week.

2.3 Preparation and Seed Planting

Turi seeds are first selected before planting, seed color, seeds are not rotten, not moldy, seeds not float when soaked in water, uniform seed size, and not shriveled. The seeds are soaked in 70% alcohol, then homogenized and left for the duration of the treatment. Seed planting is carried out inside a Laminar Air Flow.

3 Results and Discussion

Effect of alcohol soaking duration on Initial Germination Time of seeds in the medium germination can be seen in Table 1.

Table 1. Initial Germination Time (days).

Treatment	Examination			Rate
	1	2	3	
P0 (0 minutes)	0	0	0	0
P1 (5 minutes)	0	0	0	0
P2 (10 minutes)	0	0	0	0
P3 (15 minutes)	14	09	10	11
P4 (20 minutes)	10	16	13	13
P5 (25 minutes)	11	13	15	13

From the table, it can be seen that the germination time using a soaking duration of 5 and 10 minutes did not produce any germinated seeds. Seeds germinated with soaking duration of 15 minutes, 20 minutes, and 25 minutes with an average of 11 days, 13 days, and 13 days. The duration of seed soaking in alcohol affects the germination time of turi seeds. The fastest germination time occurs with a soaking duration of 15 minutes. The data obtained were not subjected to statistical analysis because the germination data obtained were less than 50%, with the rate turi seed germination ranging from 0-44% (Table 2) [7].

The low germination rate in this study may be due to the fact that turi seeds have a hard seed coat. A hard seed coat often causes seeds to experience delayed germination even after being given a scarification treatment for a certain period of time. According to [8], each type of seed from various plants has a different level of seed coat hardness, which affects the seed coat's sensitivity to water during the germination process. Turi seeds have a hard seed coat characteristic, causing physical dormancy that can create structural limitations in the germination process [1].

Seed germination in vitro is greatly influenced by the type of plant, initial seed treatment, and the composition of the culture medium. Several studies show variation in the

time of radicle emergence (the first root) as an indicator of germination success. In some plants, the germination period can vary. In dragon fruit (*Hylocereus polyrhizus*), the seeds show the fastest germination time on the 7th day after being planted in MS medium with 7.5% corn extract [9]. For lime (*Citrus aurantiifolia*), scarification treatment and application of 15 ppm GA_3 accelerated germination to 10 days, compared to the control which took 18 days. In sweet orange (*Citrus sinensis* L.), the combination of 1 ppm BAP and 2 ppm NAA produced roots on the 12th day and shoots on the 15th day [10]. Meanwhile, in *Dendrobium orchids*, *Dendrobium* seeds planted in VW (*Vacin and Went*) medium began germinating on the 21st day [11].

Effect of alcohol soaking duration on Germination Rate of seeds in the medium germination can be seen in Table 2.

Table 2. Germination Rate (%).

Treatment	Examination			Rate
	1	**2**	**3**	
P0 (0 minutes)	0	0	0	0
P1 (5minutes)	0	0	0	0
P2(10 minutes)	0	0	0	0
P3(15 minutes)	33	33	33	33
P4(20 minutes)	33	33	33	33
P5 (25 minutes)	33	33	66	44

From the table, it can be seen that the germination time using a soaking duration of 5 and 10 minutes did not produce any germinated seeds. Seeds germinated with soaking duration of 15 minutes, 20 minutes, and 25 minutes with an average of 33 %, 33 %, and 44 %.

Increasing the soaking duration up to 25 minutes showed the highest germination capacity of turi seeds, but the germination percentage in this study is still considered low because it is less than 50%, so statistical analysis cannot be conducted. Statistical analysis can be performed if at least 50% of the seeds have germinated from the total seeds sown, because if the germination value is below this threshold, it does not meet the criteria for representative data that can be tested statistically [7].

Germination of seeds is influenced by various treatments and environmental conditions designed specifically. The main factors that have been shown to affect germination success, according to Wirakusuma [12], are Plant Growth Regulators (PGRs) gibberellin (GA_3), cytokines (BAP), and auxin (NAA), which play important roles in triggering enzyme activity and accelerating seed metabolism. Application of GA_3 at 15 ppm on lime seeds increased germination vigor up to 86.7%. Mechanical or chemical scarification also has an impact, as wounding the seed coat or soaking in sulfuric acid helps water and oxygen penetrate the embryo. Scarification of castor seeds increased germination from 40% to 75% [13].

The composition of the medium also affects the culture medium [9]. MS media enriched with 7.5% corn extract has been proven to increase the germination rate of dragon fruit seeds up to 93.3%. Similarly, environmental conditions (light and temperature) for *Dendrobium orchid* seeds show an 80% germination rate under light conditions. Optimal temperature (25–28°C) and bright lighting support enzyme activity and accelerate germination [11] Additionally, Prasetyo [14] added that initial soaking treatment, by soaking seeds in warm water for 24 hours, can soften the testa and accelerate imbibition, as in tomato seeds that showed an increase in germination rate up to 90%.

Effect of alcohol soaking duration on Contaminant Percentage in the medium germination can be seen in Table 3.

Table 3. Contaminant Percentage (%).

Treatment	Examination			Rate
	1	**2**	**3**	
P0 (0 minutes)	0	0	0	0
P1 (5 minutes)	0	0	0	0
P2 (10minutes)	0	0	0	0
P3(15 minutes)	33	33	33	33
P4 (20minutes)	40	40	40	40
P5(25 minutes)	66	33	33	45

From the table, it can be seen that contamination was not found in P0, P1, and P2, and began to appear in treatments P3, P4, and P5 with soaking durations of 15 minutes, 20 minutes, and 25 minutes. Contamination in seeds with soaking durations of 15 minutes, 20 minutes, and 25 minutes was on average 33%, 40%, and 45%, respectively. Turi germination is carried out in vitro; therefore, sources of contamination must be minimized to achieve successful germination.

Increasing the soaking duration to 25 minutes showed the highest contamination in seeds, imbibiti occurred faster compared to soaking duration of 15 and 20 minutes. The speed of imbibiti increases the amount of contaminants. The entry of water into the seeds helps accelerate the growth of bacteria and fungi present in the seeds. Contaminants come from the seeds used, which were purchased online with unknown origins; for further research, certified seeds are required to minimize contaminants, so as not to interfere with the tissue. Microorganisms that attack plant tissues or cell cultures generally grow rapidly, consuming nutrients and producing toxins that can affect growth and kill plant tissue [15].

Seeds or culture medium contamination is caused by fungi and bacteria. In fungal contamination, white to black hyphae (of different types) appear on the medium or the seeds, whereas bacterial contamination is characterized by a thick liquid resembling mucus around the seeds or the medium, which is a mass of bacteria. Contamination by bacteria and fungi is a common problem in plant tissue culture. Sources of contamination can come from tools made of glass or plastic, culture media, instruments used to transfer explants to the media, plant materials used, as well as the room where explants are planted and grown.

The presence of fungi in the treatment could also be caused by the use of alcohol alone, which cannot produce 100% sterile seeds. Alcohol is effective in killing surface microbes, but it is not sufficient for fungal spores. Therefore, its use must be combined with other agents for optimal results.

Contamination is a major challenge in tissue culture, especially when using seeds. Contaminants can come from external microorganisms hidden within seed tissues. Seeds with thick and hard coats tend to harbor microorganisms in surface crevices, making them more difficult to sterilize. Turi seeds are included among seeds with hard shells. The physiological condition of old and damaged seeds will have an even higher risk of contamination because the exposed tissue makes it easier for microbes to enterasas.

Contaminants in media and seeds within tissue cultures can be anticipated through the methods and duration of sterilization. The use of a 10% NaOCl (*sodium hypochlorite*) solution for 10–15 minutes has been proven effective in reducing contamination in tomato and chili seeds. However, a prolonged duration can damage the embryo [14]. The presence of endophytic contaminants in some seeds, which harbor endophytic microorganisms that

are not eliminated by surface sterilization. Additional treatments, such as soaking in antibiotics (streptomycin or rifampicin), can help. Furthermore, using well-stored seeds will minimize contaminants.

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