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THE EFFECT OF LONG SOAKING OF SEEDS IN KNO_3 SOLUTION AND VARIATIONS IN PLANTING MEDIA ON THE GERMINATION OF CIPLUKAN (*Physalis angulata* L.) SEEDS IN VITRO

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ABSTRACT
The *Physalis angulata* L. (ciplukan), originating from the Solanaceae family, contains secondary metabolites that can be used as medicinal materials. The provision of *Physalis angulata* seeds is hindered by seed dormancy. Dormancy can be broken by employing a 0.2% w/v KNO_3 solution with a specific soaking period. This study aims to optimise the duration of the soaking period for *Physalis angulata* seed germination and the in vitro growing medium. The seeds were soaked in a 0.2% w/v KNO_3 solution for 6 hours, 12 hours, and 24 hours. The soaked seeds were planted in sterile tissue media moistened with Murashige and Skoog (MS) medium solution, a 0.2% w/v KNO_3 solution, and distilled water. Generally, the dormancy of *Physalis angulata* seeds can be broken by soaking them in a 0.2% w/v KNO_3 solution. The results of this study indicated that *Physalis angulata* seeds soaked in a 0.2% w/v KNO_3 solution for 6 hours and 12 hours, then planted in sterile tissue media moistened with the MS medium solution, exhibited an optimal germination response.

***Keywords**: Ciplukan, KNO_3, Sprouts*

ABSTRAK
Tanaman *Physalis angulata* L. (ciplukan) yang berasal dari famili *Solanaceae* mengandung senyawa metabolit sekunder yang dapat dimanfaatkan sebagai bahan baku obat. Pembenihan *Physalis angulata* terhambat oleh dormansi benih. Dormansi dapat dipatahkan dengan menggunakan larutan KNO_3 0,2% b/v dengan lama perendaman tertentu. Penelitian ini bertujuan untuk mengoptimalkan lama waktu perendaman pada perkecambahan biji *Physalis angulata* dan media tanam *in vitro*. Benih direndam dalam larutan KNO_3 0,2% b/v selama 6 jam, 12 jam, dan 24 jam. Benih yang telah direndam ditanam pada media tisu steril yang dibasahi dengan larutan media *Murashige* dan *Skoog* (MS), larutan KNO_3 0,2% b/v, dan air suling. Umumnya dormansi benih *Physalis angulata* dapat dipecahkan dengan merendam dalam larutan KNO_3 0,2% b/v. Hasil penelitian menunjukkan benih *Physalis angulata* yang direndam dalam larutan KNO_3 0,2% b/v selama 6 jam dan 12 jam, kemudian ditanam pada media tisu steril yang dibasahi dengan larutan media MS, menunjukkan respon perkecambahan yang optimal.

Kata kunci: *Ciplukan, KNO_3, Sprouts*

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INTRODUCTION

The herbal medicine has been used for a long time. Even in the modern era, herbal medicine is in great demand by the wider community. Most chemical compounds with medicinal properties are produced by plants in the form of secondary metabolites (Mainawati, 2017)[1]. Apart from being used as medicine, secondary metabolites can be used as raw materials for biopesticides, cosmetics, perfumes, and food flavourings (Julianto, 2019)[2]. Secondary metabolites do not play a direct role in plant growth and development. However, several secondary metabolites play a role in plant defence from biotic and abiotic stresses (Perangin-Angin, 2019)[3].

One of the plants that produces secondary metabolites is ciplukan (*Physalis angulata* L.), which comes from the Solanaceae family. So far, ciplukan is often considered a weed (an annoying plant), but only a few people take advantage of the benefits of this herb. According to Nuranda (2016)[4], the bioactive compounds contained in ciplukan leaves are alkaloids, saponins, and steroids; stem parts are alkaloids, saponins, steroids and flavonoids; and in fruit are alkaloids, saponins and triterpenoids. Mastuti (2020)[5] stated that ciplukan contains many secondary metabolite compounds, including withanolide, physalin, calystegine, and the tropane alkaloid nortropan which can have antibacterial, anti-inflammatory, analgesic, and antipyretic properties.

The increasing use of secondary metabolites that have the potential to be used as medicines has resulted in the need to provide raw materials in large quantities and in a short time to improve the quality of the raw materials for medicines. Techniques for increasing the production of secondary metabolites can be done through in-vitro techniques, one of which is with callus culture. Callus culture requires explants that are sterile and differentiate quickly. Providing fast-growing explants is an important stage in callus culture. One of the meristematic explants is aseptic sprouts, which are obtained from in vitro seed germination.

Information on ciplukan seed germination is still restricted. Ozaslan et al. (2017)[6] stated that ciplukan seeds (*Physalis angulata* L.) have dormancy properties so pre-planting treatment is needed to increase germination. According to Susanti (2019)[7], treating seeds before germination with 0.2% KNO_3 can increase the germination of ciplukan seeds. Information regarding the in vitro germination of *Physalis angulata* L. is still limited. Based on this, research on the germination of ciplukan (*Physalis angulata* L.) seeds is important to carry out. This research aims to determine seed treatment before germination and methods for testing the germination capacity of ciplukan seeds (*Physalis angulata* L.).

MATERIALS AND METHODS

This research was conducted at the BSF Plant Laboratory, Department of Biology, Faculty of Science & Mathematics, Diponegoro University. This research was carried out from December 2022 to May 2023. The materials used in this research were ciplukan fruit seeds (*Physalis angulata* L.), distilled water, 0.2% w/v KNO_3 solution, Murashige and Skoog (MS) basic media. The germination medium is wet tissue.

In vitro seed germination

The first step in extraction is selecting the ciplukan fruit. The ciplukan fruit used is yellowish (Chaidir et al., 2015)[8] . The seeds are then removed from the fruit, cleaned from the flesh and mucus attached so that they do not become spots to grow mushrooms, seeds are selected by looking at their physical appearance such as dense, pithy, uniform shape and size. The selected seeds are soaked in well water for six hours. The seeds that sink are then drained.

Aseptic seed germination is carried out on sterile wet tissue media. The seeds that have been selected are soaked in distilled water for 6 hours, then the seeds are soaked with a 0.2% KNO_3 solution with different soaking durations: 6 hours, 12 hours, and 24 hours. After being soaked in 0.2% KNO_3 solution with different soaking times, the seeds are planted in germination media in the form of a piece of tissue that has been moistened with 3 types of media treatment: distilled water, MS solution, and 0.2% w/v

KNO_3 solution. Each bottle of germination medium was filled with ten seeds for each replication. The seeds are then incubated at a temperature of 25℃, LED lighting of 200 lux, and humidity of 88 – 100%.

Research design

The experimental design used was a Completely Randomized Design (CRD) with a 3x3 factorial pattern. The first factor is the treatment before germination, namely the length of soaking ciplukan seeds in 0.2% w/v KNO_3 solution, namely 6 hours, 12 hours, and 24 hours. The second factor is the treatment of planting seeds in tissue media with distilled water, MS solution, and 0.2% w/v KNO_3 solution. The experiment was given ten repetitions.

Data analysis

The germination test (DB) was observed every week until the fourth week after germination (HSP). The data was analysed by Analysis of Variance (ANOVA) with a confidence level of 95% and continued by post hoc test using DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSION

The part of the plant that plays a role in reproduction is the seed. Seeds are ovules from mature and fertil flowers. Seeds before initiation of germination have a structure consisting of an embryo and food reserves. Seeds begin to germinate under favourable conditions in response to environmental stimuli such as light, temperature, soil components (especially nitrate), and the molecular mechanisms of the response have been well characterized (Nonogaki 2017)[9]. Seed germination is a change in the plant life cycle that determines success in reproduction and survival of the next generation of plants. Seed germination is considered to be the initiation of the first developmental phase in the life cycle of higher plants and is followed by post-germination growth. It is generally known that carbohydrates stored in the endosperm are so large that they affect seed size, therefore larger seeds have a better start to life and performance than smaller seeds (Gholami et al. 2009; Gunaga and Vasudeva 2011)[10]. Seed shape and size can influence water imbibition, seed moisture content and germination, and seed quality (Balkaya and Odabas 2002[11]; Cerdà and García-Fayos 2010[12]; Mandal et al. 2010). However, in certain species, small seeds do not always produce poorer seedling growth, for example medium-sized rubber seeds provide a higher percentage of germination and seedling growth than larger ones (Bahri and Saukani 2017)[13] and in soybeans, large-sized seeds have lower germination power than medium and small seeds (Yulyatin and Diratmaja 2015)[14]. Apart from size, germination is also greatly influenced by seed dormancy. Another thing that occurs in some seeds which have germination inhibitor properties, namely by producing mechanical resistance compounds against the protrusion of the radicle so that the seeds become impermeable to water or gas, for example, strawberries and tomatoes, will not germinate in fruit due to the presence of germination inhibitor substances (Hilhorst et al. 1998)[15].

Germination generally starts from water imbibition, food reserve mobilization, protein synthesis and radicle protrusion (Hasanuzzaman et al. 2013)[16]. Seed development can be maintained because seeds are a place to store energy, carbon and nitrogen for growth (Zienkiewicz et al. 2014)[17]. If the seeds do not undergo this physiological process, they can be said to be dormant. However, dormant seeds or seeds are tremendously difficult to determine because dormancy can only be measured by the absence of germination in the seeds or seeds. Moreover, there are various causes of dormancy, such as physical, physiological and hormonal dormancy contained in the seeds. Therefore, it is crucial to learn about seed dormancy and methods of breaking dormancy in seeds, to know the characteristics of seeds that must be treated with mechanical or chemical scarification and to know the effects of scarification on seeds.

Seed dormancy is the inability of seeds to germinate in an optimum environment. Dormancy was caused by the physical condition of the seed coat, the physiological condition of the embryo, or both conditions. However, dormancy does not mean that the seed is dead or cannot grow again

(Ardi, 2018)[9]. Dormancy-breaking methods can be done in various ways, including mechanical, physical, or chemical method. The chemical method is the most practical method because it is only done by mixing a chemical liquid with the seeds (Faustina et al., 2012)[10]. One chemical solution to break dormancy is KNO_3. The KNO_3 solution has also been proven to break the dormancy of several plant seeds effectively, including rice and sugar palm (Lasut, 2022)[11]. The KNO_3 solution increase the activity of growth hormones in the seeds and make the seed coat easier for water to penetrate during the imbibition process. The effect of KNO_3 caused is determined by the high and low concentrations. Initial treatment with KNO_3 solution plays a role in stimulating germination in almost all types of seeds. Soaking treatment in KNO_3 solution is also reported to activate cell metabolism and accelerate germination (Faustina et al., 2012)[10].

Figure 1. a. Ciplukan seeds are germinated in tissue media + distilled water
b. Ciplukan seeds were germinated in tissue + MS media
c. Ciplukan seeds were germinated in tissue + KNO_3 media

Table 1. Percentage of germination in the first week after planting

Germination medium	Soaking with KNO_3 (hours)		
	6	**12**	**24**
Tisu + aquades	0	0	0
Tisu + MS	10^a	40^c	0
Tisu + KNO_3	20^b	0	0

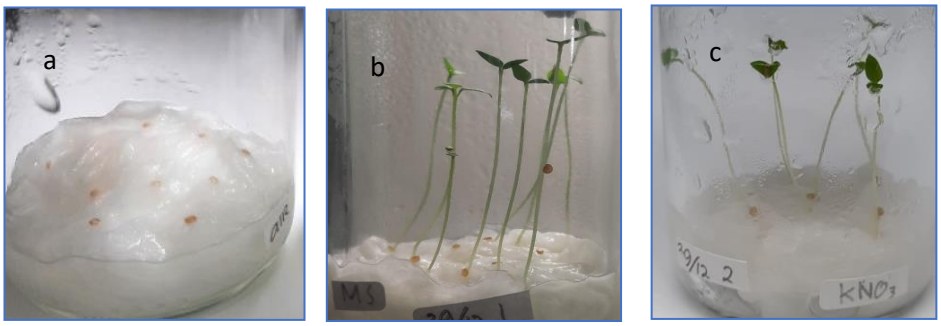

Figure 2. Growth of ciplukan sprouts one week after germination

Soaking the seeds in water will stimulate the gibberellin hormone in the embryo. This hormone will support root and tuna cell division. Air can also be used to activate hydrolysis enzymes in the endosperm layer, these enzymes will break down carbohydrates (starch) into glucose which is a respiration substrate in the embryo which will produce ATP. This ATP will play a role in cell division which causes root growth and seedling development.

Soaking with KNO_3 for twelve hours had the highest germination percentage compared to soaking with KNO_3 for six hours in the same week. The highest percentage of germination in 12 hours of soaking was produced when planted in tissue+MS media, it is suspected that in 6 hours of soaking and planted in KNO_3 media the nutritional or nutrient requirements were not met for the growth of the sprouts. In general, the growth of ciplukan sprouts requires additional nutrition from KNO_3 and MS.

Table 2. Percentage of ciplukan germination based on the length of time the seeds are soaked in sterile wet tissue media

Germination medium	Soaking with KNO3 (hours)			P < 0,001
	6	12	24	
Tisu + aquades	0,00 ± 0,00[c]	0,00 ± 0,00[c]	0,00 ± 0,00[c]	
Tisu + MS	**30 ± 0,21[a]**	29 ± 0,18[a]	7 ± 0,11[b]	
Tisu + KNO_3	11 ± 0,09 [b]	5 ± 0,07[b]	3 ± 0,05[a]	

Note: the mean number followed by the same letter in the same column indicates that it is not significantly different based on the ANOVA test at the 5% confidence level

The statistical test results showed that the planting medium in the form of tissue moistened with MS solution was the treatment that caused the highest germination of ciplukan seeds compared to the other two treatments. This shows that the presence of nutrients (nutrients, vitamins, and amino acids) in MS media increases the germination of ciplukan seeds. Germination of ciplukan seeds in the tissue media treatment moistened with KNO_3 solution was lower than in the tissue + MS media treatment. Allegedly, the nitrogen element in KNO_3 is a nutrient that plays a role in the growth of embryos in seeds. However, small ciplukan seeds (± 1 mm) not only need nitrogen, but also vitamins and amino acids contained in MS. Tissue media moistened with distilled water did not seem sufficient to support ciplukan seed germination. It is suspected that ciplukan seeds are non-endospermic seeds. Endospermic seeds are a part of seeds that do not grow well because the endosperm formed during seed development has been absorbed into the cotyledons causing the seeds to form a thick structure. This causes the reserve food content to be insufficient to stimulate embryo growth.

The soaking time of 6 and 12 hours in tissue + MS planting media was the treatment that caused the highest germination of ciplukan seeds compared to the soaking time of 24 hours. The soaking time of 6 hours in tissue media + KNO_3 was the treatment that caused the highest germination of ciplukan seeds compared to the other two treatments (12 and 24 hours). Based on observational data, it is known that soaking ciplukan seeds in 0.2% w/v KNO_3 solution is quite effective in breaking seed dormancy. Halimursyadah (2020)[12] stated that soaking seeds in a KNO_3 solution can stimulate enzyme activity to renovate food reserves in the seeds. The KNO_3 solution is known to have a stimulatory effect on seed germination. When soaking ciplukan seeds in KNO_3 solution, after the imbibition process occurs the activity of hydrolysis enzymes will increase, thereby increasing other metabolic activities such as respiration, one of the products of which is energy in the form of ATP which is needed for germination and growth of sprouts (Muhar, 2015)[13]. Soaking ciplukan seeds for 6 and 12 hours can soften the skin of ciplukan seeds, making it easier for water and oxygen to enter the seeds. The chemical treatment aims to make the hard seed coat permeable to water during the inhibition process. (Rahmatica, 2020)

CONCLUSION

Soaking ciplukan seeds in a 0.2% KNO_3 solution can break the dormancy of ciplukan seeds by 22%. Seeds soaked in 0.2% KNO_3 solution for 6 hours and planted on tissue + MS media produced an optimal number of sprouts, namely 30%.

There needs to be variations in the immersion treatment carried out for research the effect of soaking on ciplukan germination, for example by the difference in

temperature of the water used for soaking to determine the effect of broader immersion on germination and growth of ciplukan seed sprouts

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