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Effect of Potassium Nitrate (KNO_3) on Indonesian Konjac Productivity

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Abstract Indonesian konjac (IK in brief), also known as *Amorphophallus muelleri* Blume in Latin, is a wild plant growing in several places in Indonesian archipelago. The tuber of IK plant contains a compound called glucomannan which has high economic value since it can be used as a raw material in many industries such as medicine, cosmetic, paper, textile, synthetic rubber, and filming industries. Due to this economic value, this study was carried out in order to increase the productivity of glucomannan and bring IK into mass cultivation. For this purpose, the objective of this study is to increase the productivity of IK tubers by increasing the speed of seeds gemination process and by decreasing the dormancy period. Meanwhile, bringing IK into mass plantation will be put as a package of campaign program to educate people. To speed up the germination process and to decrease the dormancy, the method used in this study period is by improving the soaking process of IK seeds using KNO_3 solution. Its effects were investigated using a completely randomized design (CRD) with three treatments, namely, concentration of the solution, soaking time, and plant age. Then, data were collected and analyzed statistically using general linear model, analysis of variance and Duncan's multiple range test. The results indicate that soaking in that solution has a significant effect on shortening the time period for seeds to germinate. Its optimal effect was reached for 3,000 ppm of concentration with soaking time 3 hours at 14^{th} days after plantation (DAP). Moreover, in terms of dormancy period, that solution has reduced from 5-6 months to 2-4 months. These findings were significantly support the effective use of KNO_3 solution to answer the objective of research. The germination period has been reduced from 3-6 months to around 14 days. To the knowledge of the authors, based on the literature used in this study, these are unprecedented findings. Therefore, hopefully, it could contribute to the development of konjac-based industries and to the literature of konjac particularly Indonesian konjac.

Keywords Analysis of Variance, Dormancy Period, Duncan's Multiple Range Test, Germination Rate, Soaking Process

1. Introduction

1.1. Background of the Study

Historical background at a glance

This paper deals with a phenological study of Indonesia konjac (*Amorphophallus muelleri* Blume), a wild plant with promising economic potential but still mysterious in breaking the dormancy period and speeding up the germination process. For brevity, in what follows we refer to that plan as IK. As a wild plant, it is not an indigenous Indonesian plant. According to Jansen et al. (1996), it is originally from West Africa. More specifically, it was from the paleotropics West Africa. It came (more precisely, was brought) to Indonesia during the era of Dutch colonization hundreds of years ago. **IK tuber has many benefits in various industries like for food and**

pharmaetica industries (Dwiyono and Djauhari, 2019). However, its cultivation began only when Japan occupied Indonesia. Nowadays, it grows worldwide in the tropical and subtropical zones of the paleotropical kingdom comprising the tropical areas of Africa, Asia and Oceania (excluding Australia and New Zealand). In Indonesia, in particular, it spreads out in Sumatera, Java, Madura, Bali, Celebes, Flores and Lombok.

Phenological background

IK is one of tuber plants. It belongs to the *Araceae* family and *Monocotyledon* class. The three main products of this plant (from highest to lowest economic value) are the tubers, the bulbils, and the seeds. See Figure 1 for illustration.

IK tuber contains a compound called glucomannan. It is this compound that has high economic value and since very recently it has become an export commodity to several countries such as Japan, Taiwan, Korea, China, Netherland, and other European countries. This compound has many benefits for industry as well as world food security program. In industry, such as medicine, cosmetic, paper, textile, synthetic rubber, filming industries, glucomannan is usually used as raw material for their products. For example, in Japan medical industry, it is the raw material to produce dietary food such as "*konyaku*" and "*shirataki*." These foods contain a lot of fiber suitable to support dietary program. They may increase food digestibility, reduce blood cholesterol level and bring down obesity. Interestingly, as mentioned in Bo et al. (2013), it contains anti-*human immunodeficiency virus* (anti-HIV) compound. Furthermore, see Jansen et al. (1996), with appropriate processing IK tuber can be turned into a substitute to traditional food. This will be important during food crisis. Jansen et al. (1996) also mention that IK plant is also known as indigenous traditional medicine to treat some diseases such as dysentery, cholera, digestive disorder and rheumatic. These are the phenological background of this study.

1.2. Status of the Konjac Plant in the Market

For production purposes

In nature, IK plant grows well in lowland with altitude 100 meters above sea level (masl) as well as in upland (1000 masl). It has the best growth under 50 t0 60 percent sunlight with (i) the soil acidity of 6 to 7.5, (ii) sandy, loose and compost soil, (iii) rainfall of 1000 to 1500 millimeters every year, and (iv) air temperature of 26 to 30^o Celsius. In this environment, Indonesian farmers have experienced the increase of yearly export value of IK tubers from 1999 to August 2003. It was consecutively 199,828 tones (267,104 USD), 181,055 tones (245,488 USD), 179,597 tones (317,675 USD), 125,747 tones (264,132 USD), and 266,719 tones (385,995 USD). Those yearly productions were still lower than foreign demand. This year 2020 alone, according to the Ministry of Agriculture, Republic of Indonesia, only 12 % of demand have been fulfilled. Therefore, there is an urgent need to increase the productivity of IK tubers.

(a) (b) (c)

Figure 1. (a) Farmer showing 5.5 kg IK tubers harvested after 2-3 years of plantation, (b) bulbils, and (c) cob of fruits which produces seeds

For agriculture

The unbalance condition between foreign demand and local production of IK tubers offers a challenging opportunity for those who involve in the agricultural industry to invest in IK plantation. In these regards, a piece of surprising news came from a European Community agricultural research center. Very recently Dr. Alberto Forte, Director of the DASAM, Department of Sustainable Agriculture at IEMEST (https://www.iemest.eu/en/), Palermo, Italy, has mentioned in his email to the authors that konjac is one of the interests of European Community in developing and diversifying agroindustry in Mediterranean area.

As we all know, Mediterranean area is a sub-tropic region with four seasons whereas konjac is a tropical plant. This shows how konjac has become an interest of worldwide community.

1.3. Literature Review

To increase the productivity of tuber, breaking or reducing the dormancy period and speeding up the germination process becomes a priority in IK cultivation. This is a phenology-based problem. Actually, research on this problem is not new. Since the last two decades phenology is an acive research. This is reported, for example, in Chmielewski, et al. (2004), Cleland, et al. (2007), Atkinson, et al. (2012), Jin, et al. (2013), Workie and Debella (2018). However, these authors' concern is on the phenology of fruit, trees, field crops, plant, and vegetation in general. In this study the focus is on the phenology of IK. **The fruit spadic of IK plant produces betweem 100-500 fruits and each fruit contain 2-4 seeds (Dwiyono, 2019).**

The topic on how to increase the germination rate and to shorten the dormancy period has received special attention from the researchers on various types of plants. For example, Hilton and Bitterli (2006), Abdelgadir, et al. (2012) and Dostatny, et al. (2015) have reported their results on germination of *Avena fatua*. Meanwhile, Moravcová and Dostálek (1989) and Eslami (2011) have focused on germination of *chenopodium album*, and Ohadi, et al. (2009) and Derakhshan, et al. (2014) on canary grass, Gardarin, et al. (2011) and Alshallash (2018a) on that of weed, and very recently Bhatt, et al. (2019) on germination behavior of perennial halophyte of Arabian deserts. **Hasan et al. (2017) stated that reduction of germination percentage and delay of wheat seed caused by the higher of salt concentration in media.** Other researchers, Baskin and Baskin (1988), have described the behavior of seeds germination of herbaceous plant. Similar study on seeds germination was conducted by Ohadi, et al. (2009) on *phalaris minor* and *poa annua* and by Alshallash (2018b) on oat. Other example given in Grubišić and Konjević (1990) is about the interaction of light, nitrate and alternating temperature in promoting the germination of dormant seeds of weed species. **The various physiological and biochemical processes like water stress can reduce growth and productivity of hararghe coffee in Eastern Ethiopia (Wegari and Amin, 2020)**.

The above literature review led to fix objective of this study. It is to increase the productivity of glucomannan. For this purpose, breaking the dormancy period and speeding up the germination process are the main concerns. Traditionally, after being harvested, IK seeds from parent tree cannot immediately germinate and then grow because they experience long dormancy period about 5-6 months Bian, et al. (2013). To accelerate the seeds growth from its formation, see Simón, et al. (2018), it needs to break the dormancy period.

In order to understand how to increase the seeds germination rate, a three-factor experiment under completely randomized design was conducted at Dramaga Research Field, Bogor Agricultural University, Indonesia. This experiment was deigned under the following assumption: "Seeds germination rate is considered as a function of (i) concentration of KNO_3 solution, (ii) soaking time, and (iii) plant age." In laboratory level, the seeds germination rate (in %) was observed using 5 levels of concentration, 4 levels of soaking time, and 8 levels of plant age.

The use of KNO_3 was inspired by the previous studies on the productivity of several plants. For example, Bian, et al. (2013) that KNO_3 treatment under 6,000 ppm concentration with 24 hours soaking time may produce unsweetened-red palm seed by 65.33% compared with control (36.00%) on 22 weeks after planting (WAP). Copeland and McDonald (1999) gave an important remark on the effectiveness of soaking pine walnut into KNO_3 solution. At 1,500 ppm concentration, it has significantly accelerated the germination. Other example was showed by Qaderi and Cavers (2000). They concluded that higher concentration level of KNO_3 up to 200 ppm may trigger the germination of seeds from *eragrotis curvula* species. Meanwhile, Gashi, et al. (2012) have remarked that the application of 500 ppm and 1,000 ppm KNO_3 concentration with 14 hours light soaking time and 10 hours dark on *onopordium acanthium* L seeds produced the germination rate of 66.6% and 88.2%. It is higher than the control (41,8%).

The previous studies mentioned above were the inspiration why in this study KNO_3 was used to fasten the IK seeds germination process and shorten the dormancy period. An advantage of this chemical is that it enables to give additional O_2 useful for accelerating the seeds respiration and trigger the conversion of seed carbohydrate compound into simple sugars that will be used as energy source for germination. Then, it will be decomposed into nitric acid and K element. Nitric acid has the role attenuate the seed shell to facilitate oxygen in the water to come into the seed while K element will speed up

water absorption. Thus, the germination runs faster. According to Ruttanaruangboworn, et al. (2017) and Mayer and Poljakoff-Mayber (1989), the lack of oxygen is one of the reasons why seeds cannot grow immediately. **According to Dwiyono et al. (2019), the gibberellic acid in 200-300 part permillion (ppm) soaking time 12 hours and at 12 day after planting (DAP) observation can upgrade of germination rate process in IK seeds to 4.33 % and 12.67 % respectively than the control (0.33 %). During the dry season the IK plants dry out or enter a dormant period which time between 5-6 months (Dwiyono, 2020)**

1.4. Research Problems

The successful studies from the previous researchers mentioned above led the authors to conduct an experiment in order to investigate the use of KNO_3 solution in increasing the productivity of IK plant. More specifically, to investigate the effect of the use of KNO_3 solution in seeds soaking process to speed up the seed germination process and to shorten the dormancy period. Faster germination process will increase the production. It is so with shorter dormant period.

In that experiment, a CRD with tree treatments, i.e., concentration of KNO_3 solution, soaking time, and plant age was used. Then, data collected from that experiment were then analyzed in two steps. At the first step analysis of variance (ANOVA) was used to test whether concentration, soaking time and plant age in terms of days-of-plantation (DAP) have different effect (Gomez and Gomez in (1984)). If the result is significantly different, at the second step a multiple comparison was studied using Duncan's multiple range test (DMRT) originally introduced by Duncan (1955). DMRT is one of the most common methods used in comparing treatment means. It summarizes the way in finding several significant differences with increasing values (Dafaallah (2019)). At the end, treatments have the same effect will be revealed. To support this analysis, all statistical computations were performed with the help of Statistical Analysis System (SAS).

The results of this experiment are very promising. The effect of KNO_3 concentration and soaking time is significant. The germination rate increases for certain level of concentration but then decreases after that level. It is so with soaking time. It is worth noting that more than 3 hours of soaking leads to lower germination rate and higher concentration will cause toxic to seed germination process. The condition giving highest germination rate is also identified. The details will be given in the rest of the paper which is organized as follows. The next section presents the method and materials followed in Section 3 by the results issued from GLM, ANOVA and DMRT. Finally some conclusions in Section 4 will end this presentation.

2. Method and Materials

Germination is a nature-made process of transforming seeds into young plants. Only by understanding this process, speeding up the germination process and shortening the dormancy period can be possible. The main objective of this research is to have a better understanding of the effect of soaking the IK seeds in KNO_3 solution and soaking time on the seeds germination rate for different plant age. To answer these objectives, in this section the methods, materials, statistical design and analysis are presented.

2.1. Methods

First, a laboratory experiment was conducted using CRD with three treatments, namely the concentration of KNO_3 solution in part-per-million (ppm), the soaking time (in hour), and the plant age in days after planting (DAP). Then, from this experiment, data were collected. This was followed by data preparation, data analysis and statistical analysis. The results are used to verify whether the objectives have been achieved or not.

2.2. Materials

During the experiment, the materials used were IK tubers and seeds collected from Dramaga Research Field, KNO_3, aquadest, and compost fertilizer. And, the equipment consists of calipers, analytical weighing scale, rulers, measuring cylinder, nursery tray, wool, thread, paper label and bamboo.

2.3. Statistical Design or Design of Experiment

To achieve the objective, five levels of concentration (0 ppm as the control treatment, 1000, 2000, 3000, and 4000 ppm) and four levels of soaking time (0, 3, 6, and 12 hours) were used. Then, the germination rate was observed on the 14^{th}, 21^{st}, 28^{th}, 35^{th}, 42^{nd}, 49^{th}, 56^{th}, and 63^{rd} DAP.

In this experiment the seeds used were the ones harvested 45 days before planting. They were divided into 40 lots were prepared and each lot containing 100 seeds, was put into cross stitch cloth. The seeds were then soaked in aquadest to clean. After cleaning, they were soaked in KNO_3 solution. Some 800 seeds were soaked in the solution of the first level of concentration. The same number of seeds were soaked in the second, third, fourth and fifth levels. And, at each level of soaking time, 1000 seeds were soaked. After soaking process, the seeds were washed with aquadest to remove KNO_3 residual on the seeds surface. Then, they were planted in plastic jar containing washed-quartz sand.

An experiment unit was defined as one plastic jar. These units were arranged randomly. The observation was

done at every 7 days starting from age 14 to 63 DAP. The data observed from normal germinated seeds in each experimental unit were recorded and converted into percentage. The criterion of being normal germinated seed is when radicle becomes a perfect shoot and plumule has formed perfect leaf.

2.4. Statistical Analysis

Based a CRD with several treatments mentioned above, to test whether the concentration and the soaking time have different effect or not for each DAP, a multiple range test was employed. For this purpose, first, a general linear model (GLM) and ANOVA were used to analyze the data. If different effect occurs in different DAP, based on Duncan (1955) as suggested by Gregory (1965) and Dafaallah (2019), then Duncan's multiple range test (DMRT) was used. In these regards, all statistical computations were performed using SAS System.
The GLM for ANOVA under CRD in DAP is,

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{ijk} + \omega_l + \gamma_{kl} + (\alpha\omega)_{il} + (\beta\omega)_{jl} + (\alpha\beta\omega)_{ijl} + \varepsilon_{ijkl}$$

Here Y_{ijkl} is the germination rate using the *i*-th level of KNO_3 concentration, *j*-th level of soaking time, *k*-th replication and *l*-th DAP, and,

(i) μ is the overall mean
(ii) α_i is the effect of the *i*-th level of KNO_3 concentration
(iii) β_j is the effect of the *j*-th level of soaking time
(iv) $(\alpha\beta)_{ij}$ is the interaction of KNO_3 concentration and soaking time
(v) δ_{ijk} is the random component of KNO_3 concentration, soaking time and replication
(vi) ω_l is the effect of the *l*-th level of DAP
(vii) γ_{kl} is the random component of replication and DAP
(viii) $(\alpha\omega)_{il}$ is the interaction of KNO_3 concentration and DAP
(ix) $(\beta\omega)_{jl}$ is the interaction of soaking time and DAP
(x) $(\alpha\beta\omega)_{ijl}$ is the interaction of KNO_3 concentration, soaking time and DAP
(xi) ε_{ijkl} is the overall random component

By using this model, the results will be presented and discussed in the next section.

3. Results and Discussion

Table 1 in the Appendix summarizes the effect of soaking into KNO3 solution on seeds germination rate (in %) for each concentration and soaking time observed in terms of DAP. The letters a, b, and c are used to indicate whether the results of DMRT are significant or not for Type I error of 5%. For a DAP, the same letter in a column means no different effect.

In our experiment, the first germination appears on 14th DAP. We learn from this table that;

1. The germination rate on the 14th, 21st and 28th DAP with concentration 4,000 ppm is in general significantly higher than the control. But, the observations on the 35th until 63rd DAP did not show significant difference. This indicates that KNO3 has been significantly served to accelerate the germination process.
2. In general, in terms of concentration, the highest germination rate occurred at 3,000 ppm but not significantly different at 4,000 ppm and beyond, except on the 14th DAP where the highest occurred at 4,000 ppm. On the other hand, in terms of soaking time, the highest germination rate occurred for 3 hours. Thus, to a certain extent, higher concentration will accelerate the germination process. However, overdoses of concentration will lead to a poisoning condition.
3. The soaking time of 6 and 12 hours did not give significant effect. In general, soaking more than 3 hours leads to lower germination rate for all concentrations. This might happen since longer soaking time will cause toxic to seed germination.
4. Observation on 21st DAP showed that there was no effect of the combined treatments concentration and soaking time. Meanwhile, the concentration of 3,000 ppm produced the highest result. Interestingly, the effect caused during the initial treatment (0 hour soaking time) was not always followed by the same result overtime. This could be caused by the effect of KNO3 compound which had been absorbed by the seed during nursery period.
5. Observation at 28th, 35th, and 42nd DAP showed relatively similar effect compared to those observed at 21st DAP where concentration of 3,000 ppm and 3 hours soaking time which tended to give the highest result.
6. Observation at 49th DAP showed that for 0 hour soaking time at all 5 levels of concentration, the same results were produced except for concentration of 2,000 ppm. This concentration has significantly produced the lowest result, while the highest result was produced with 3,000 ppm. However, there was no significant difference between the application of 0, 1,000 and 4,000 ppm.
7. Soaking time of 6 hours in 5 levels of concentration did not show significant difference on the germination rate. Similar results were obtained for 3 hours soaking time except for soaking with 1,000 ppm which produced the lowest value. Meanwhile, the highest germination rate was produced at 3,000 ppm and 3 hours.
8. From all those treatments, it can be said that 3 hours soaking time and 3,000 ppm concentration gave the

best result when the plant age was 14 days. After that day, the effect of KNO_3 decreased and disappeared after 49th DAP. The study showed that the same germination rate will be obtained after 63rd DAP no matter whether KNO3 was used or not.

9. It seems that the use of potassium nitrate in this study was on the right way since, according to Ruttanaruangboworn, et al. (2017), nitrate compound can reduce the amount in the metabolic regulatory process involving nicotinamide adenine dinucleotide phosphate (NADP) in a particular reaction on glucose metabolism.
10. Barba-Espín (2012) stated that KNO3 solution at concentration 2,000 ppm in dark condition and temperature 15-30oC can trigger the seeds germination of lepidium virginicum, eragrotis curvula, polypogon monspelliensis, agrostis, and sorghum halepense. This paper shows that the use of that solution to trigger the seeds germination of IK has similar effect.
11. At the concentration 3,000 ppm and soaking time for 3 hours the use of KNO3 tends to accelerate the seeds germination process of IK since the dormancy period has been reduced to 2-4 months. This is a significant result compared with the result in Jansen, et al. (1996) which stated that the dormancy period of IK is 5-6 months.

4. Conclusions

In this research, KNO_3 was applied in soaking process to accelerate the seeds germination of IK and to shorten the dormancy period. The effect of its concentration and soaking time was significant. The germination rate increased when the concentration moved from 1000 ppm to 3000 ppm but then decreased for 4000 ppm. The highest germination rate occurred at concentration 3,000 ppm on 14^{th} to 63^{rd} DAP. Furthermore, in terms of soaking time, 3 hours soaking time with 3,000 ppm concentration and 14^{th} DAP gave the best result. It is worth noting that more than 3 hours of soaking leads to lower germination rate and higher concentration will cause toxic to seed germination process.

The study in this paper indicates that KNO_3 has been significantly served to accelerate the germination process. To a certain extent, higher concentration will accelerate the germination process. However, overdoses of concentration will lead to a poisoning condition. Longer soaking time will also cause toxic to seed germination.

This study is unprecedented. If the previous researchers showed that the use of that KNO_3 solution can trigger the seeds germination of several plants, this paper shows similar effect when it was used to trigger the seeds germination of IK. More precisely, it can be said that 3,000 ppm concentration and 3 hours soaking time gave the best result when IK plant age was 14 days. Moreover, the dormancy period has been reduced from 5-6 months to 2-4 months.

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APPENDIX

Table 1. Effect of soaking into KNO_3 solution on seeds germination rate (in %) using different concentration and soaking time observed in terms of DAP

Observation (DAP)	Concentration (ppm)	Soaking time (hours)			
		0	3	6	12
14	0	0.67b	1.67a	0.00b	0.00b
	1000	0.67b	2.67a	0.33b	0.00b
	2000	0.33b	6.00a	1.33b	0.00b
	3000	0.67b	5.33a	1.00a	0.67b
	4000	6.33a	0.67b	1.33a	0.33b
	Mean	1.73ab	3.27a	0.80bc	0.20c
21	0	11.00a	10.00a	1.00a	2.00a
	1000	4.00a	14.00a	7.00a	8.00a
	2000	6.00a	16.00a	8.00a	4.00a
	3000	15.00a	20.00a	9.00a	7.00a
	4000	17.00a	7.00a	6.00a	4.00a
	Mean	10.80a	13.27a	6.40b	4.87b
28	0	39.00a	42.00a	16.00a	27.00a
	1000	37.00a	39.00a	34.00a	34.00a
	2000	30.00a	49.00a	36.00a	29.00a
	3000	50.00a	49.00a	29.00a	37.00a
	4000	48.00a	41.00a	30.00a	30.00a
	Mean	30.73a	43.93a	29.00b	31.47b
35	0	65.00a	56.00a	40.00a	45.00a
	1000	59.00a	53.00a	55.00a	56.00a
	2000	44.00a	61.00a	55.00a	48.00a
	3000	68.00a	71.00a	50.00a	61.00a
	4000	62.00a	64.00a	47.00a	53.00a
	Mean	59.67a	61.20a	49.40b	52.60b
42	0	74.00a	73.00a	53.00a	59.00a
	1000	71.00a	67.00a	65.00a	65.00a
	2000	58.00a	74.00a	66.00a	57.00a
	3000	78.00a	81.00a	64.00a	68.00a
	4000	73.00a	74.00a	61.00a	71.00a
	Mean	70.87a	73.67a	61.87b	63.93b
49	0	78.33a	80.30ab	61.67a	69.67ab
	1000	80.67a	70.00c	71.67a	73.67ab
	2000	64.67b	79.00ab	69.33a	67.67b
	3000	82.00a	84.00a	68.33a	72.00ab
	4000	78.67a	76.67b	68.67a	77.00a
	Mean	76.87a	70.00a	67.93b	72.00b
56	0	87.00a	84.67ab	67.33a	71.67b
	1000	85.67a	75.33c	75.67a	74.33ab
	2000	65.00b	84.67ab	71.67a	74.00ab
	3000	82.00a	88.33a	76.33a	76.67ab
	4000	79.67a	79.00bc	73.00a	82.67a
	Mean	79.87ab	82.40a	72.80c	75.87bc
63	0	91.00a	89.00ab	75.67a	76.00b
	1000	86.67a	79.33c	77.67a	77.00b
	2000	70.67b	88.00ab	77.67a	78.33b
	3000	85.00a	92.33a	82.00a	82.00ab
	4000	84.33a	81.33bc	77.00a	85.33a
	Mean	85.33ab	86.00a	78.00c	79.80bc

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