Effect of Gibberellic Acid on Konjac Seeds Germination:

Evidence from Data Analytics

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Abstract

This paper aims to find an optimal combination of gibberellic acid concentration (C) and soaking duration time (T) in order to speed up and to improve the productivity of konjac (*Amorphophallus muelleri* Blume) germination process. For this purpose, a laboratory experiment was carried out with five levels of C and four levels of T. For each combination of C and T, 300 seeds were planted in three germination trays. And by using completely randomized design, in each tray, 100 seeds were planted. On the day right after a number of days after planting (D) which consists of seven levels, the germination rate is then recorded. By using data analytics method, we conclude that the optimal combination of C, D and T is C = 200 ppm, D = 7 days and T = 6 jam. Meanwhile, for a given T and D, concentration has no effect on germination rate. It is worth noting that (i) the experiment was conducted at room temperature under controlled humidity level, and (ii) according to the knowledge of the authors, this is an unprecedented study where Gibberellic acid is applied on konjac.

Keywords: Breaking seed dormancy, food and cosmetic industry, food security, konjac germination process, konjac productivity

1. Introduction

Konjac (*Amorphophallus muelleri* Blume) is a Monocot wild herb. It can be found in ASEAN countries such as Cambodia, Indonesia, Malaysia, Philippines, and Vietnam as a wild herb. It can also be found in China and Japan where the word "konjac" was originally introduced (konyaku in Japanese). It is a tuber crop which belongs to the Araceae family. In Indonesia, people call it "Iles-iles" and it has been traditionally cultivated for many years. For traditional people, konjac is to increase family income. As mentioned in Sugiyama & Santosa (2008), it has been consumed as traditional food and has a high potential for agroforestry due to rich glucomannan content.

Glucomannan is commonly found in the form of low-digestive carbohydrate which has wide application in many industries such as food, textile, synthetic rubber, electric isolator, and cosmetic industries. It is also used as a traditional medicine of various diseases like dysentery, cholera, cancer, respiratory disorders, and rheumatism. Currently, dried-corms of Indonesian konjac are exported to countries like China, Japan, and Korea. See Jansen et al. (1996) for further discussion on this kind of business. Thus, konjac has high economic value either in the food industry or cosmetic industry.

Due to this economic value, many researches have been carried out on (i) improving the value of konjac, and (ii) domestication program of this wild herb. For example, a very recent research result reported in Santosa et al. (2016a) had remarked that agronomic manipulation on flowering konjac is necessary to enhance seed production. It is then followed by Santosa et al. (2016b) to increase the production of seeds. All these works motivate us to study the optimal condition to speed up konjac germination process and increase its productivity. This is the goal of this paper.

Improving the productivity of konjac is a crucial problem since the seed has a long dormancy period, i.e., between 2 and 3 months. Its size is of 0.4-0.7 cm width and 0.9-1.3 cm length. And the color varies from white to gray to

blackish depending on the level of maturity. Each cob contains 100-400 berries and each berry contains 2-3 seeds. So, according to Santosa et al. (2016a,b), each plant produces 200-1200 seeds depending on the size of the cob and weight of the tubers planted. Physiologically, the seeds are protected by a thick skin pulp and the embryo is considered not ripe enough to germinate. That is why the dormancy period is long. To shorten this period or to speed up the germination process, as remarked in Santosa et al. (2006), the most common treatment is chemical treatment using gibberellic acid (GA3). However, the activity of GA3 hormone depends on the concentration and duration of immersion. And, according to Murni et al. (2008), the concentration of GA3 to some extent can spur germination but the excessive concentrations might act as an inhibitor.

The germination process is the first step of any food engineering activities. Until present, there are a lot of time and works spent on improving the germination process. For example, Afolayan et al. (1997) were doing research to improve the germination of everlasting flower, Asra (2014) in germination and vigority of Calopogonium blue, Murni et al. (2008) in germination and vegetative growth of duku, Mistian et al. (2012) in germination of betel nut, and Wang et al. (2012) who worked in germination for heteromorphic seeds. Now, we can say that the main purpose of this research is to find an optimal concentration and duration of immersion to produce higher germination rate in a shorter period of time.

Gibberellic acid (GE3) is a plant hormone which is widely used to stimulate seed germination by activating enzymes especially α -amylase. It hydrolyzes starch to sucrose and glucose in producing energy during seed germination. From the literature, we learn that GA3 has been widely used to trigger cell division and elongation, shorten the seed dormancy period, accelerate germination, and trigger a parthenocarpic or seedless fruits. To mention some, here are some previous findings. Murni et al. (2008) had reported a good effect of GE3 on duku (Lansium dookoo), Putri & Herawan (2008) on sandalwood, Kurniawati & Hamim (2009) on carambola fruit, Doaigey et al. (2013) on date palm, Astawa et al. (2015) on Balinese table grape to extend the length of the fruit bunch size, and Zhang et al. (2017) on watermelon. We can also see in Afolayan et al. (1997) a discussion on the effect of GE3 on an everlasting flower, Santosa et al. (2006) on elephant foot yam, Saleh & Wardah. (2010) on palm sugar, Mistian et al. (2012) on betel nut, Harahap et al. (2015) on rubber plant to increase the shoot growth, and Sunmonu et al. (2016) on bean and maize.

However, the effect of GA3 depends on plant species. For example, see Jansen et al. (1996), the effectivity of the GA3 on the seed of A. muelleri which mainly contains glucomannan is still unknown. On the other hand, according to Saleh & Wardah (2010), GA3 increase seed dry weight of areca nut (Areca catechu L) seed. Meanwhile, see Murni et al. (2008), on the other seed such as duku (Lansium dookoo Griff) fruit, GA3 accelerates the seed germination. Thus, as remarked in Emamipoor & Maziah (2014), in general, GE3 is usually used in breaking the seed dormancy.

In this research, GA3 was applied to stimulate konjac seed for the first time. It was carried out to evaluate the effect of GA3 under different soaking concentration and duration of seed germination. For this purpose, a laboratory experiment was conducted to find the optimal combination of concentration (C), soaking duration time (T) and observation day right after a number of days after planting (D) to get higher germination rate in a shorter period of time. In this experiment, the temperature was set to room temperature and the humidity level was controlled at 65-70%. Meanwhile, C has five levels, T has four levels, and D is of seven levels.

In the next section, we begin our discussion with the methodology of the experiment, data collection, and data analysis. Later on, Section 3 presents the results of data analysis followed with a discussion. To close this presentation, in the last section (Section 4) concluding remarks which include the direction of future research will be highlighted.

2. Method

The seed was selected from mature konjac cob at University farm of Bogor Agricultural University, Indonesia. The pulp was carefully removed inside flowing water and only shrinking seeds in the water were used in this experiment. Then, the seeds were dried in the room temperature for one night before treatment. The size of the seeds is 0.4-0.7 cm width and 0.9-1.3 cm length.

Konjac seeds were distributed in different levels of C, T, and D using complete randomized design with three replications. To find the optimal combination C, T, and D which will give the higher germination rate in a shorter period of time, here is the design.

(i) C is divided into five levels namely, 0 (control), 200, 300, 400 and 500 ppm where 0 ppm means that the seeds are soaked in distilled water.

- (ii) T has four levels, that is, 0 (control), 6, 12 and 18 hours where '0' hour of soaking was made by single dipping for about a second. After the treatment, as suggested in Santosa et al. (2006), the seed was kept in room temperature for one night to facilitate drying.
- (iii) D is of seven levels, i.e., 7, 12, 18, 24, 30, 36 and 42 days.
- (iv) In each replication and each observation period, 100 seeds were used.

The treated seeds were planted in germination trays inside the greenhouse at the Faculty of Agriculture, National University, Indonesia. The planting condition is as follows.

- (i) Planting media was mixed of soil, sand and compost fertilizer at ratio 1:1:1 (volume/volume).
- (ii) Seeds were planted at about 2 mm in depth.
- (iii) The temperature during the planting period ranged between 35 and 40 °C with relative air humidity between 65 and 70%.
- (iv) Irrigation water was applied twice a day, i.e., at 7 am in the morning and at 5 pm in the evening.

Data on germination rate are then collected 7, 12, 18, 24, 30, 36 and 42 days after planting (D). Therefore, during this experiment 4800 seeds were used in total. The number of germination seeds was counted. A seed was considered as successfully germinates when active bud arose, irrespective of the bud position.

To analyze the collected data, the method of data analytics is used to see the pattern that exists in the dataset. The interpretation and conclusion will be drawn based on that pattern.

3. Results and Discussion

Data obtained in this experiment are summarized in Table 1 at the end of this paper. The number recorded in this table represents the average germination rate of three replications. For example, at D = 7, C = 200 and T = 12, the recorded number is 1.67. This number is the average of three germination rates each of which comes from one of the three germination trays.

3.1 Effect of Concentration

For each pair of D and T, it is hard to say that C has a significant role. We cannot say that the higher the concentration the higher the germination rate. As evidence, we show in Figure 1 the effect of C in the horizontal axis on the germination rate in the vertical axis for D = 7 and D = 12. The three colored lines; blue, red and green represent T = 6, 12 and 18, respectively.



Figure 1. Effect of concentration on germination rate

As we will see in Figure 2, the effect of concentration is not as strong as D and T. In this figure, the horizontal axis is D while the vertical axis is the same as in Figure 1, i.e., germination rate. In Figure 2(a), which represents C = 0 ppm, there are four colored curves; purple, blue, red, and green. They correspond to T = 0, 6, 12 and 18. We see clearly that for all levels of D there is almost no effect of T at C = 0. However, and interestingly, the four colored curves have a sigmoid model. And, it is worth noting that this model also appears in the other levels of C. This complies with the model in growth theory. Furthermore,

1. Figure 2(b) until Figure 2(e) represent germination rate as function of D at C = 200, 300, 400 and 500 ppm. In these figures, three colored curves; blue, red and green (representing T = 6, 12 and 18) are plotted. Like in

Figure 2(a), Figure 2(b) shows that there is almost no effect of T on germination rate. This means that for C = 200 ppm, the best choice is T = 6 for all levels of D.

- 2. For C = 300 ppm and low levels of D (7, 12, 18), see Figure 2 (c), the best choice is T = 6 (red) but T = 18 (green) dominates the other levels of T for D greater than 18. This means that at C = 300 ppm the seeds that soaked during (i) 6 hours have highest germination rate until the 18^{th} day, and (ii) 18 hours have highest germination rate after the 18^{th} day.
- 3. Now, at C = 400 ppm in Figure 2(d), whatever the level of D there is no different whether T = 6, 12 or 18.
- 4. Finally, for C = 500 ppm in Figure 2(e), green curve (T = 18) dominates blue and red curves (T = 6 and 12) for all levels of D except D = 7. However, this level of concentration is the most costly compared to the lower levels.

From the above results, if our concern is on high germination rate (vertical axis) with high speed of germination process (small levels of D) and low cost (low levels of C), the best choice is;

- (i) C = 200 ppm and T = 6 (blue curve), or
- (ii) C = 300 ppm and T = 12 (red curve).

However, in general, the best performance is given by the combination of C = 500 ppm and T = 18 (green curve).





(e) Concentration 500 ppm Figure 2. Germination rate versus D for 3 all levels of T

3.2 Dormancy Period

It is also important to note that the experiment shows a new perspective in the dormancy of Indonesian konjac seed. As shown in Table 1, the seeds in the control tray started to germinate at D = 12. This means that dormancy less likely exists in Indonesian konjac seeds. In other words, the dormancy rate is low. This phenomenon of the Indonesian konjac seed has been discussed in Santosa et al. (2016b). However, as noted in Sumarwoto (2005), a further study is required if one wants to evaluate whether the seed of Indonesian konjac from different agro-ecology exhibit different dormancy rate.

Table 1. Germination rate (%) under different GA₃ concentration (ppm) and soaking duration (hour)

Observation day D	Concentration C				
		0	6	12	18
7	0	0	0	0	0
	200	-	4	1.67	2
	300	-	1	5.33	1.33
	400	-	2	3.67	2.67
	500	-	1.67	1.33	3
12	0	0.33 d	0.33	0.33	0.33
	200	-	8.33	4.33	6.00
	300	-	4.00	12.67	8.00
	400	-	8.33	7.67	7.33
	500	-	8.00	4.67	10.67
18	0	3.00 d	3.00	3.00	3.00
	200	-	10.67	7.33	10.00
	300	-	8.33	20.67	23.00
	400	-	13.33	16.33	12.00
	500	-	10.67	10.67	22.67

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	0	17.00	17.00	17.00	17.00
	200	-	24.33	17.00	20.00
24	300	-	18.33	27.00	35.67
	400	-	25.33	25.67	23.00
	500	-	18.33	27.33	37.00
	0	59.67	59.67	59.67	59.67
	200	-	43.00	42.33	47.33
30	300	-	48.33	55.67	66.00
	400	-	53.33	53.67	52.00
	500	-	39.33	51.67	60.00
	0	80.00	80.00	80.00	80.00
	200	-	66.00	66.67	66.67
36	300	-	73.33	78.00	83.67
	400	-	75.33	73.00	75.67
	500	-	63.33	72.33	76.00
	0	87.00	87.00	87.00	87.00
	200	-	79.00	80.00	87.00
42	300	-	84.67	86.00	90.33
	400	-	84.33	87.00	90.67
	500	-	80.67	85.33	88.67

4. Concluding Remarks

Due to its economic value, many researches on konjac have been carried out to improve its quality and productivity, and to widen its domestication program. Under this spirit, this paper aims to find the optimal condition to speed up konjac germination process and increase the germination rate. In this research, GA_3 was applied to stimulate konjac seed. Then, we evaluate the effect of GA_3 under different soaking concentration (C) and soaking duration (T). The germination rate was observed and counted D days after planting. After data were collected, data analytics method was used to understand the pattern that exists in the dataset. We then interpret this pattern and draw the conclusion.

4.1 In Terms of Germination Rate

It is worth noting that,

- 1. For each pair of D and T, it is hard to say that the higher the concentration the higher the germination rate. And in general, the effect of concentration is not as strong as D and T.
- 2. For C = 200 ppm, the best choice is T = 6. However, for C = 300 ppm, the seeds that soaked during 6 hours have the highest germination rate until the 18^{th} day. After this day, soaking period of 18 hours is the best.
- 3. In general, the best combination is C = 500 ppm and T = 18. However, if our concern is on high germination rate with high speed of germination process and low cost, the best choice is C = 200 ppm and T = 6 or C = 300 ppm and T = 12.

4.2 In Terms of the Dormancy Period

We conclude that,

- 1. The productivity of konjac is a crucial problem since the seed has a long dormancy period between 2 and 3 months.
- 2. The experiment in this research shows that the dormancy rate of Indonesian konjac seeds is low, i.e., 12 days.

4.3 Future Research

To evaluate whether the seed of Indonesian konjac from different agro-ecology exhibit different dormancy rate a further study is required. This is a problem for future research.

To close this presentation, it is worth noting that for all soaking period, the growth model has a sigmoid pattern. This pattern appears in all levels of concentration.

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