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ผลของระดับความเข้มข้นของโคลชิซินและระยะเวลาในการแช่ต่อการกลายพันธุ์แบบโพลิพลอยด์ในข้าวฟ่าง (*Sorghum bicolor* L. Moench) 5 สายพันธุ์

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บทคัดย่อ: การศึกษาครั้งนี้เป็นการศึกษาผลของระดับโคลชิซิน ระยะเวลาการแช่ และสายพันธุ์ข้าวฟาง (*Sorghum bicolor* L. Moench) 5 สายพันธุ์ ต่อการกลายพันธุ์แบบโพลิพลอยด์ โดยใช้การทดลองแบบ 4x5x5 แฟคทอเรียลในแผนการทดลองสุ่มตลอดโดยสมบูรณ์(CRD) ซึ่งมี 3 ปัจจัย คือ ระดับความเข้มข้นของโคลชิซิน (0, 01, 0.2 และ 0.3) 5 ระยะเวลาของการแช่ (0, 12, 24, 48 และ 72 ชั่วโมง) และ 5 สายพันธุ์ข้าวฟ่าง (Cowley, Rio, IS23585, Keller และ Wray) ผลการศึกษาพบว่า ปฏิสัมพันธ์ร่วมระหว่างระดับความเข้มข้นของโคลชิซิน ระยะเวลาในการแช่ และสายพันธุ์ของข้าวฟ่าง มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P \leq 0.05$) เมื่อระดับความเข้มข้นของโคลชิซินเพิ่มขึ้น และระยะเวลาในการแช่ที่นานขึ้น ส่งผลให้อัตราการรอดชีวิตของข้าวฟ่างมีค่าลดลงและแตกต่างกัน แต่ทำให้ต้นกลายพันธุ์แบบโพลิพลอยด์เพิ่มขึ้น แตกต่างกันในแต่ละสายพันธุ์ ข้าวฟ่างพันธุ์ Cowley ที่ระดับการใช้สารโคลชิซินที่ระดับ 0.1 เปอร์เซ็นต์ แช่นาน 48 และ 72 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 3.3 และ 2.3 เปอร์เซ็นต์ตามลำดับ การใช้สารโคลชิซินที่ระดับ 0.2 เปอร์เซ็นต์ แช่นาน 24 และ 48 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 4.1 และ 3.2 เปอร์เซ็นต์ ตามลำดับ และการใช้สารโคลชิซินที่ระดับ 0.3 เปอร์เซ็นต์ แช่นาน 24 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 4.0 เปอร์เซ็นต์ สำหรับสายพันธุ์ Rio การใช้สารโคลชิซินที่ระดับ 0.1 เปอร์เซ็นต์ แช่นาน 48 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 2.2 เปอร์เซ็นต์ การใช้สารโคลชิซินที่ระดับ 0.2 เปอร์เซ็นต์ แช่นาน 24 และ 48 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 5.2 และ 2.6 เปอร์เซ็นต์ ตามลำดับ การใช้สารโคลชิซินที่ระดับ 0.3 เปอร์เซ็นต์ แช่นาน 24 ชั่วโมง ให้ต้นกลายพันธุ์แบบโพลิพลอยด์ 2.8 เปอร์เซ็นต์ สำหรับสายพันธุ์ IS23585, Keller และ Wray ไม่มีความแตกต่างทางสถิติของการใช้สารโคลชิซินในระดับต่างๆ และระยะเวลาของการแช่ต่างๆ อย่างไรก็ตาม หากพิจารณาความถี่ของโอกาสเกิดการกลายพันธุ์แบบโพลิพลอยด์มากที่สุด คือ ที่ระดับความเข้มข้นของโคลชิซิน 0.2 เปอร์เซ็นต์ แช่นาน 48 ชั่วโมง ซึ่งเป็นความเข้มข้นและระยะเวลาการแช่ที่เหมาะสมมากที่สุดที่ทำให้เกิดการกลายพันธุ์แบบโพลิพลอยด์

คำสำคัญ: ข้าวฟ่าง กลายพันธุ์ สารโคลชิซิน โพลิพลอยด์

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Effects of Colchicine Concentration Level and Soaking Period to Induce Polyploid Mutation on Five Forage Sorghum (*Sorghum bicolor* L. Moench) Cultivars

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Abstract: This study was to investigate the effect of the colchicine concentration level, soaking period, and five forage sorghum (*Sorghum bicolor* L. Moench) cultivars on the occurrence of polyploidy. The 4x5x5 factorial arrangement in Completely Randomized Design (CRD) was employed through 3 factors: 4 colchicine concentrations level (0, 0.1, 0.2 and 0.3%), 5 soaking periods (0, 12, 24, 48 and 72 hours) and 5 forage sorghum cultivars (Cowley, Rio, IS23585, Keller and Wray). The results revealed that there was a significant difference among colchicine concentrations level, soaking periods, and sorghum cultivars; when colchicine concentration level increased and soaking period increased, the survival rate of sorghum decreased variously but occurrence of polyploidy chromosome increased differently among cultivars. Cowley sorghum soaking in the colchicine concentration at 0.1% for both 48 and 72 hours had mutation polyploidy 3.3 and 2.3 % respectively. Cowley sorghum soaking in the colchicine concentration at 0.2% for both 24 and 48 hours had mutation polyploidy 4.1 and 3.2 % respectively and soaking in the colchicine concentration at 0.3% for 24 hours had mutation polyploidy 4.0%. While, Rio sorghum soaking in the colchicine concentration at 0.1% for 48 hours had mutation polyploidy 2.2 % and soaking in the colchicine concentration at 0.2 % for 24 and 48 hours had mutation polyploidy 5.2 and 2.6 % respectively. For IS23585, Keller and Wray sorghum cultivars weren't significant different effect of the colchicine concentration level and soaking period. However, considering the frequency of the occurrence of seedling having the polyploidy chromosome soaking in the colchicine concentration of 0.2% for 48 hours was the most appropriate concentration and period or duration for the occurrence of the plant with the polyploidy chromosome.

Keywords: Sorghum, Mutation, Colchicine, Polyploid

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Introduction

Sorghum is one of important corps of the world ordering from rice, wheat, corn and barley. It is a drought resistant plant. Its seeds is consumed and used as an animal feed, while its stalk is used as a fresh or fermented food for ruminants. Sorghum is a plant that can leave the stump after cultivating which can be utilized as a feed for animal as well (Maiti, 1996; Poosamart, 2008; Pholsen *et al*., 2001; FAO, 2008). Regarding the special properties of sorghum, there should be the breeding for the better properties. Sorghum is a plant that imported from other countries which this type of sorghum can be used its stalk as a food for ruminants (Suksri and Pholsen, 1999). Inducing the mutation by using the chemical mutagens such as colchicine is one of many methods that can make the chromosome mutation (Newcomer, 1941; Rao and Suprasanna, 1996; Shao *et al*., 2003) resulted in the structural change or chromosome aberration leading to changing the genetic code of plant (Luca, 2000; Dhooghe *et al*., 2011) or the increasing of chromosome (Daker, 1967; Rao and Suprasanna, 1996; Wu and Mooney, 2004; Xu *et al*., 2010) which is called polyploidy mutation. This chromosome help to increase the size of plant such as the width and length of leave, to produce the biomass productivity (Levine, 1945; Gu *et al*.,2005; Mujib, 2005; Ghaffari, 2006; Yang *et al*., 2006), to be darker for the green of the leave (Wei *et al*., 2007) and to be higher for the stalk (Glowacka *et al*., 2010). Consequently, the investigation of inducing mutation of sorghum through the use of colchicine to produce the polyploidy chromosome is very interesting. This can be a choice for breeding the sorghum in terms of finding out an appropriate concentration level and the soaking time which help can increase the polyploidy chromosome. With the expectation, it will affect to the size of meristem cells which result in the bigger size of other parts of plant such as size of leave, the height of stalk and the diameter of stalk, and improve the growth ratio of sorghum together with the increase of biomass productivity of the mutant sorghum which may be useful for the breeding of sorghum.

Materials and Methods

This study employed the 4x5x5 factorials arrangement in completely random design (CRD) as research design for experimenting three factors: firstly, the four concentration level of colchicine (0%, 0.1%, 0.2% and 0.3%), secondly, the soaking period (0, 12, 24, 48, 72 hours), and finally, the five types of sorghum; *Sorghum bicolor* L. Moench (Cowley, Rio, IS23585, Keller and Wray). The rich seeds were selected to be soaked in the different concentration levels of colchicines

0%, 0.1%, 0.2% and 0.3%) which is the pure colchicine of 95% for different times of 0, 12, 24, 48 and 72 hours. For performing the experiment, the researchers grew all seeds in the same day in order to stimulate the mutation and change the embryogenesis (Dhooghe *et al*., 2011). The 100 prepared seeds were grew in the plastic tray with 104 holes full of mixed soil (sand: black husk at the ratio of 1 : 1). When the sprout was grew for 15 days after emergence, the researchers collected the data about the survival ratio, the number of the sprouts having polyploidy chromosome, and the chlorosis by using the sampling of 10% of the survival sprouts or choose all of them when the sprouts have the survival ratio less than 10% (Figure 1). Then, the sprouts were moved to be grown in the 10 x 12 inch black plastic bag waiting for checking the chromosome by microscope when they reach 2 - 3 weeks after emergence (WAE) through the method of Lertprasertrat *et al*. (1992) Chaiyasut (1989). The phase contrast microscope was used to take a picture for counting the numbers and measuring the size of chromosome via the computer package (Motic images plus 2.0) (Figure 2). The analysis of variance was employed to statistically analyze the data through the factorial arrangement in completely randomized design and was tested for detecting the difference at the level of $P \leq 0.05$ via Duncan's Multiple Range Test (DMRT).

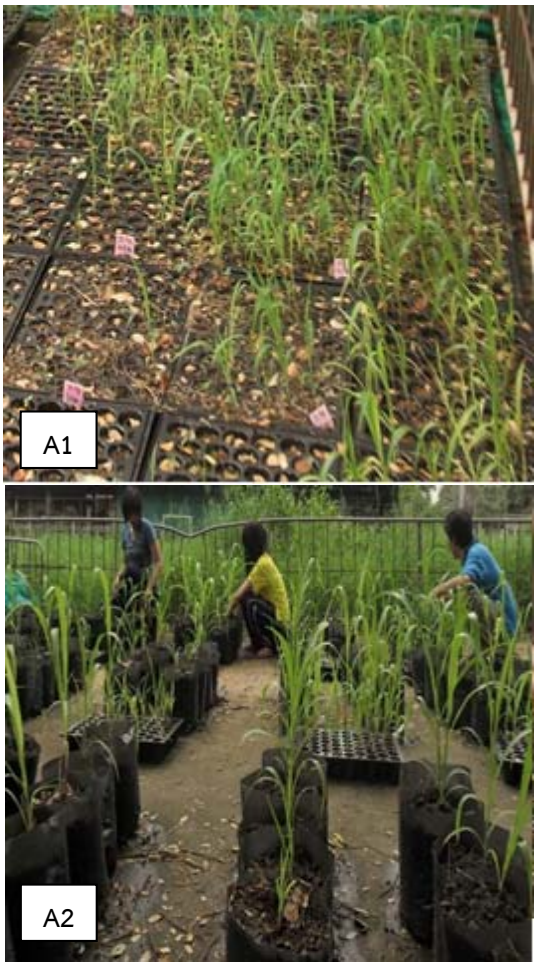

Figure 1. A1) sorghum seeding at 14 days after emergence and A2) transplanting to plastic bag

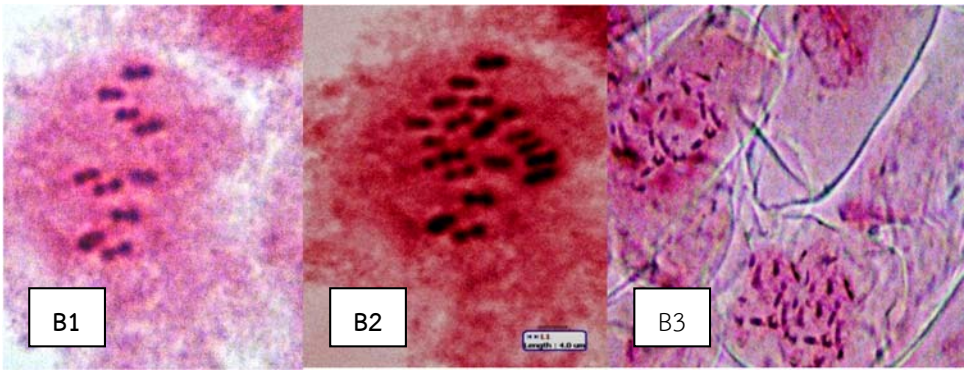

Figure 2 B1**:** chromosome number of diploid and B2-B3: chromosome number of polyploidy

Results and Discussion

From the experiment on survival ratio, the number of the sprouts having the polyploidy chromosome and the chlorosis after soaking the seeds of five types of sorghum in the four different concentration level of colchicine and the five different time to stimulate the mutation and the embryogenesis was presented in Table 1 below.

The analysis of variance of the five types of sorghum revealed that there was a relationship in all aspects. The use of variance estimation showed that there was a significant difference at the level of $P \leq 0.01$ for the squared average of the survival rate (70.4), the number of sprouts having the polyploid (8.5) and the number of the chlorosis sprouts (2,312.4). In the experiment of breeding through the use of mutation technique using chemical, radius affecting the DNA, the lack of deficiency and the aberration, there were found that the difference types of plant affected to the mutation emergence at the high level (Lamseejan *et al*., 2001). This might be due to the fact that the characteristic of each plant is different. This corresponded with the study of Chen *et.al*. (2001) who found that the Zaojing 26 (Japonica rice) and the Zhou You 6 (Indica rice) stimulating to be mutant by using colchicine at the concentration levels of 50, 75 and 100 milligram per liter had the different survival and fruitlessness rates regarding the chemically different elements of flour and carbohydrate of seeds. Furthermore, this might be affected by the different size of rice seed. The weight of 1,000 seeds of Zaojing 26 rice seed and the Zhou You 6 rice seed were 22.8 and 28.4 grams, respectively. The different size of seed affecting to the chemical absorption stimulates the different mutation. The organic chemical elements in the coat and pericarp and the chemical absorption cause the different mutation. In this experiment, it was found that the factor regarding the types of sorghum differently affected to the mutation

Table 1 The analysis of variance of sorghum varieties on concentration of colchicine and soaking period

Source	df	Plant survival (%)		Polyploidy plant (%)		Chlorosis plant (%)	
Replication	2	11.8		1.8		343.9	
Varieties (A)	4	70.4	**	8.5	**	2,312.4	**
Concentration (B)	3	86,754.4	**	4.6	*	7,448.4	**
soaking hour (C)	4	5,0429.9	**	8.5	**	8,260.3	**
Var*Conc(A×B)	12	38.2	**	1.8	ns	389.4	**
Var* soaking hour (A×C)	16	39.9	**	3.3	**	431.4	**
Conc* soaking hour (B×C)	12	7,806.1	**	3.9	**	3316.9	**
Var*Conc* soaking hour (A×B×C)	48	23.1	**	1.6	ns	192.7	ns
Error	198	5.4		1.5		154.4	

ns = there is no significant difference

* and ** = there are significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively

and the chlorosis. This might be caused by the different size of seeds of each type at the different weight per 1,000 seeds which the Cowley, Rio, and IS 23585 had the weight at 22.5 - 26.8, 20.5 - 25.4 and 21.09 - 29.40 grams, respectively (Poosamart, 2008). The Keller sorghum had the weight per 1,000 seeds at 17 grams (Chainat Research and Development Feed Center, 2015), while the Wray had the weight per 1,000 seeds during 20 – 26 grams (Lertprasertrat *et al*. 1992). In addition, the Cowley and Rio sorghums having the mutation and giving the sprouts have the polyploidy chromosome resulted in the white seed coat and had the weight per 1,000 seeds 22.5 - 26.8 and 20.5 - 25.4 grams, respectively. For the other types of sorghum which did not produce the polyploidy chromosome is ones having brown and red seed coat. Zhunsuwan (2005) reported that sorghum having brown and red seed coat have the higher amount of tannin than those having white seed coat. Tannin had the property of stopping the digestion of protein. Khacharoen (1999) stated that the different color of seed coat had the different tannin elements which results in the different organic chemical of each type of sorghum affecting to the absorption of colchicine and also the different mutation. In the current study, the types of sorghum clearly affected to the change ratio of survival rate, the sprouts having polyploidy chromosome and the chlorosis sprouts. To clearly explain the results of the experiment, there should be the consideration of two factors affecting the survival rates, which were the increase of polyploidy chromosome and the emergence of the chlorosis sprouts.

The ratio of polyploidy chromosome emergence in sorghum

The change and increase of the mitotic chromosome doubling of polyploidy chromosome of five types of sorghum soaked in the colchicine at the four different of concentration levels with the five different times showed that it was the influence of the co-interaction value of two and three factors. Moreover, the results also revealed that only two types of sorghum; Cowley and Rio, could produce the polyploidy chromosome. On the other hand, the IS23585, Keller and Wray sorghums could not produce the polyploidy chromosome. Regarding the critique on types of sorghum (Table 2), the number of the sprouts having the polyploidy chromosome of the Cowley had no co-interaction between the concentration level of colchicine and the soaking period. When considering the influence of each factor separately, it was found that the concentration levels of colchicine did not significantly produce the number of sprouts having the polyploidy chromosome and the influence of the soaking period factor was highly significant difference for producing the number of sprouts having the polyploidy chromosome as well (Table 2). However, the concentration levels of colchicine and the soaking periods resulted in the ratio of emergence of the sprouts having polyploidy chromosome. The concentration level of colchicine at 0.1% with the soaking period for 48 and 72 hours produced one sprout having the polyploidy chromosome which is CM14803, CM=Cowley mutation. The concentration level of colchicine at 0.1% with the soaking period for 48 hours produced three sprouts (3.3%) and one sprout (2.3%), respectively. The concentration level of colchicine at 0.2% with the soaking period for 24 and 48 hours produced two sprouts having the polyploidy chromosome (CM22406 and CM22414) (4.1%) and the other two sprouts (CM24803 and CM24812) (3.2%). The concentration level of colchicine at 0.3% with the soaking period for 24 hours produced one sprout having the polyploidy chromosome (CM32404) (4.0%).

The polyploidy chromosome emergence rate of the Rio sorghum comparing to the control group of the concentration at 0% with the soaking period for 0-72 hours. The higher concentration level and the longer period of soaking reduced the survival rate. The concentration level and the soaking period highly caused the change of embryogenesis and probably lead to the polyploidy mutation. The results illustrated that the concentration level of colchicine at 0.1% with the soaking period of 48 hours had similar proportion of survival rate, the number of sprouts having polyploidy chromosome and the chlorosis when

Table 2. The results of concentration levels of colchicine and soaking period to induce polyploidy chromosome of five sorghum varieties

Concentration level of colchicine (%)	Soaking (hours)	Sprout having the polyploidy chromosome (%)				
		Cowley	Rio	IS23585	Keller	Wray
0	0	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	12	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	24	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	48	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	72	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
0.1	0	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	12	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	24	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	48	3.3[ab] (n=1)	2.2 [ab] (n=1)	0.0 [b]	0.0 [b]	0.0 [b]
	72	2.3[ab] (n=1)	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
0.2	0	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	12	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	24	4.1[a] (n=2)	5.2 [a] (n=1)	0.0 [b]	0.0 [b]	0.0 [b]
	48	3.2[ab] (n=2)	2.6 [ab] (n=2)	0.0 [b]	0.0 [b]	0.0 [b]
	72	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
0.3	0	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	12	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	24	4.0[a] (n=1)	2.8 [ab] (n=1)	0.0 [b]	0.0 [b]	0.0 [b]
	48	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
	72	0.0[b]	0.0 [b]	0.0 [b]	0.0 [b]	0.0 [b]
F-test	Conc. (A)	ns	ns	ns	ns	ns
	Soaking (B)	**	ns	ns	ns	ns
	A×B	ns	ns	ns	ns	ns
CV (%)		244.63	309.76	0.0	0.0	0.0

ns = not significant difference, ** there is a highly significant difference ($P \leq 0.01$)

[ab] the different letter in the same row refers to there is significant difference ($P \leq 0.05$)

compared to the control group (concentration level of colchicine at 0% with the soaking period of 0-72 hours). The Rio sorghum emerged from the interaction between the concentration level of colchicine and the soaking period of the sorghum's seeds. The higher concentration levels together with the longer soaking period

reduced the number of the regular sprout. The concentration level and soaking period result the highest embryogenesis may lead to the polyploidy chromosome mutation. The results showed that the concentration level of colchicine at 0.1% with the soaking period for 48 hours produced one sprout having the polyploidy chromosome (RM14807) (2.2%). The concentration level of colchicine at 0.2% with the soaking period for 24 and 48 hours produced one and two sprouts having the polyploidy chromosome (RM24805, RM24811) (5.2% and 2.6%, respectively). The concentration level of colchicine at 0.3% with the soaking period for 24 hours produced one sprout having the polyploidy chromosome (RM32407) (2.8%). This was in accordance with the report from the study of Yang *et al*. (2006) who proposed that using colchicine at the concentration level of 0.1% with the soaking time at 24 and 48 hours stimulated the grape to produce the polyploidy chromosome at 6.8% and 3.0%, respectively. The results from the study of Glowacka *et al*. (2010) also corresponded with the results of this study mentioning that the use of colchicine at the concentration level of 626 and 1252 µM stimulated the Miscanthus grass to produce the polyploidy chromosome at 2.01% and 1.75%, respectively. The results of this study was also similar with the study of Wei *et al*. (2007) who reported that the Lespedeza Formosa feed bean soaking with the colchicine at the concentration level of 0.01% – 0.20% for 36 hours could survive and produce the polyploidy chromosome at 44.4% and at the concentration level of 0.1% with the soaking period for 36 hour is the most suitable time that produce the highest number of sprouts having polyploidy chromosome. The results of this study was followed with the study of Jonathan *et al*. (2008) who stated the use of colchicine at the concentration level of 0.2% resulted in the Japanese barberry (*Berberisthunbergii* var. atropurpurea) producing the tetraploid chromosome induction efficiency at 10.0%. The study of Gu *et al*. (2005) also agreed with the results of this study by proposing that the use of colchicine at the level 0.01 - 0.30% with the soaking time for 24 - 96 hours stimulated the jujube (*Zizyphus jujube* Mill. cv. Zhanhua) producing the polyploidy chromosome as 1% (5.00 ± 2.35%) and 3% (8.57 ± 2.30%), respectively. The results from this study also revealed that sorghum mutation and have polyploidy chromosome were Cowley and Rio, both are white sorghum, while the other three sorghums namely IS 23585, Keller and Wray which have the red or brown husk sorghum could not be mutant and produce the polyploidy chromosome after soaking with colchicine, which might be due to the biochemical

characteristics of the husk and size of the seed of sorghum.

Conclusion

In conclusion, it was found that the co-interaction between the concentration level of colchicine, the soaking period and the different types of sorghum were significant different. The interaction showed that the increase of the concentration levels of colchicine and the soaking period resulted in the increase of survival rate, the emergence of polyploidy chromosome sprouts and different in each cultivars. When considering the frequency of chance of highest polyploidy chromosome emergence, it was found that the concentration level of colchicines at 0.2% with the soaking period for 48 hours is the most suitable procedure for Cowley and Rio sorghum.

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