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Triploidy Induction in Banana Shrimp, *Fenneropenaeus merguiensis* (De Man, 1888) Using 6-Dimethylaminopurine

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Abstract: Triploidization is a method of producing sterile organisms for aquaculture. Eggs of *Fenneropenaeus merguiensis*, banana shrimp were shocked with 6-dimethylaminopurine (6-DMAP) at a certain concentrations and various time durations. The experimental shocks had successfully produced triploid for all the treatments where the triploidy rate was relatively high. Shock of 6-DMAP at 200 µM of 2 minutes and 400 µM of 10 minutes concentration produced highest mean chromosome numbers of 118±12.00 and 118±14.80 respectively. The highest percentage of triploidy rate (100%±0.00) was achieved at 200µM of 6-DMAP for 20 minutes. There was a significant different ($P<0.05$) in triploidy rates between the groups of different concentrations and the time duration ($P<0.05$). Hatching rate was correlated with the dose potency and time duration. Higher concentration produced low hatching rate and vice versa. The study had shown the effectively of 6-DMAP in inducing triploidy in *F. merguiensis* despite of the low hatching rate. Further optimization of triploidy induction using 6-DMAP may be possible to enhance the result.

Key words: *Fenneropenaeus merguiensis* · Banana shrimp · Chemical shock · 6- Dimethylaminopurine

INTRODUCTION

Shrimp culture has become one of the most leading crustacean productions and also is becoming more important in developing countries both for domestic consumption as well as for export. It has also significantly contributed to the socio-economic conditions of the fishing community [1]. A review done by [2] stated that the application of biotechnology to penaeid prawn aquaculture is increasing important to overcome the growing problem of diseases, slow growth and control reproduction. Chromosome manipulation is one of the methods being applied in aquaculture to improve growth of aquaculture species such as polyploidization [3]. Triploidization is now well known as a method of producing sterile organisms for aquaculture. This induction is an alternative way to improve cultured animals and has been commercialized in the production of oyster since 1985 [4]. Organisms with triploid set of chromosome have three homologous chromosome set and are found simultaneously in both wild and cultured and populations [5]. Sterility may offer better growth for the aquaculture organisms after sexual maturation because energy is not diverted to reproduction [6]. Research on triploid molluscs and fish had been done more widely compared to crustaceans' species although few of the species has economic importance [4]. Moreover, marine shrimp farming is the most aquaculture sector in the world [7]. Therefore, in recent years, some progress has been made in triploid production in shrimps. The study conducted by [4] showed that a triploid shrimp exhibited greater growth during mature stage compared to diploids.

The population biology of banana shrimp, *Fenneropenaeus merguiensis* has been studied intensively for over 20 years in Australia [8]. Aquaculture of *F. merguiensis* had been known in substituting the

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culture *Penaeus monodon* in southeast Asia and Australia due to the lower quality of *P. monodon* post-larvae growing out [9]. These days, *F. merguiensis* is one of the major crustaceans cultured in Thailand [10]. *F. merguiensis* is an important species for commercial fisheries in the Indo-Pacific region however, this species has been ignored by modern shrimp farmers because it has slow growth rate [11-13] and does not grow as fast as *P. monodon*, the major culture species [14]. Triploidy may have potential to improve the growth, because energy would not be diverted for reproductive transferred to solely on somatic growth in sterile species [15]. Therefore, triploidy induction in this species may have potential to produce more sterile offspring to grow fast. A successful study can help increase the shrimp culture technology in terms of shrimp production in future. The production of sterile shrimp for ponds grow out has the potential to protect the wild population from being invaded by the superior genetic engineered shrimps in case of escape from the culture environment [16]. The main aim of this study was to induce triploidy in *F. merguiensis* through chemical shock using 6-dimethylaminopurine and to determine the triploid and hatching rate post shock.

MATERIALS AND METHODS

Broodstock Maintenance: The gravid shrimps at stage 4 of ovarian maturation measuring length of 17 cm and weight of 50 g approximately, were collected from the wild populations from fishermen at Pulau sayak, Kedah (lat: 5°40'0"N and long: 19'60"E). The shrimps were acclimated for few hours to the new environment before to put in hatching tanks. The gravid shrimp were kept in a spawning tank of 500 L at a ratio of three shrimps per tank. The optimum water salinity and temperature for the spawning was maintained at 29°C and 33ppt.

Collection of Fertilized Eggs: The spawning initiation of egg release lasted about 10 minutes and eggs for triploid inducing were taken as time zero [6, 17].

Triploid Induction by Chemical Shock: The eggs collected, counted and placed in specially constructed containers for treatment. The eggs were treated with 200µM, 400µM and 600µM 6-DMAP for 10 minutes, 15 minutes and 20 minutes for each treatment to induce triploidy. 90 numbers of egg samples were used in each treatment applied shock. After the treatment, the eggs were removed from the treatment containers and were well-rinsed [6; 17]. The eggs then were transferred to hatching tanks filled with disinfected seawater for hatching provided with aeration.

Ploidy Detection: The detection of triploidization was done through chromosome evaluation [6; 17]. A total of 30 eggs from each replicate of each treatment were taken for the study. About three hours after spawning, eggs were treated with 0.05% colchicines for 80 minutes at room temperature (25°C). Then eggs were placed in a hypotonic solution of 0.1M potassium chloride (KCl) for 25 minutes and were fixed using five changes of Carnoy fluid (methanol-acetic acid, 3:1). For preparation of ploidy level, the eggs were placed on a slide, individually treated with acetic acid and were then broken by slight blows and left to air dry and finally the samples were stained with 10% giemsa. The number of chromosomes were counted using Karyotyping Video Test Software 3.1 [17]. The number of diploid and triploid eggs was recorded where a diploid (2n) shrimp contains 88 number of chromosome while a triploid (3n) shrimp contents extra number of chromosomes with the maximum of 132 numbers of chromosomes ranging between 89 - 132 number chromosomes [17]. The rate of triploidy was determined by dividing the number of triploid eggs with the total number of eggs used. Advance microscopes *Nikon eclipse 80i* was used to photograph the chromosome pictures[17].

Estimation of Hatching Rate of Treated Eggs: Hatching rate was calculated by counting the number of hatched larvae and non-hatched eggs after 12 to 16 hours of treatment. To determine the hatching rate, the number of nauplius were counted and divided by the total number of eggs in each treatment.

Statistical Analysis: The result were presented as mean ± Standard Deviation (SD) using Microsoft Excel and statistical testing to compare differences between the concentrations of 6-DMAP and the durations of shocking were carried out using one-way and two-way ANOVA analysis of variance (P<0.05) followed by Tukey test

RESULTS

Ploidy Detection: The effect of 6-DMAP on chromosome was shown in Table 1. Shock of 6-DMAP at 200 µM at 20 minutes duration produced the highest mean of chromosomes of 118± 12.00 and 118± 14.80 respectively. Different s hock did produce different numbers of

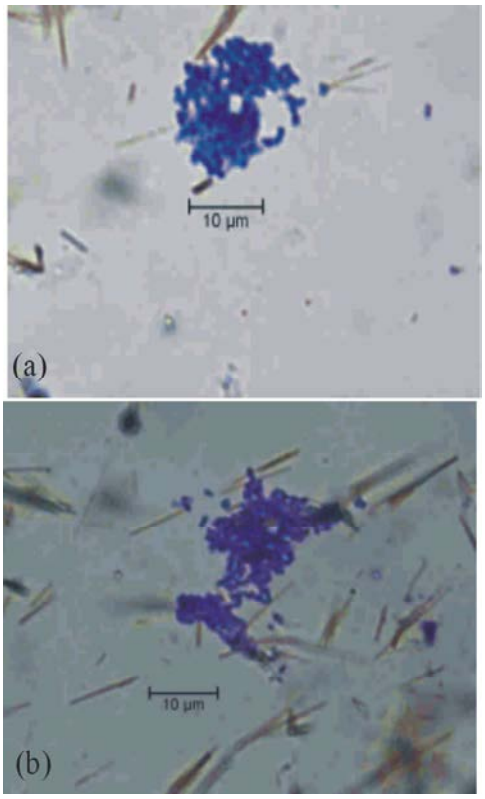

Fig. 1: Pictures of triploid chromosomes identified from 600µM concentrations treatments of 6- DMAP of F. merguiensis eggs.

chromosomes correlation with duration. The extreme numbers of triploid chromosomes which are more than 132 in some of the eggs were also observed in the present study and were considered as aneuploidy in which abnormal diploids or triploids were produced. The result also shows that some eggs produced different number of chromosomes in the same egg and this situation is called mosaicism. Figure 1 (a) and (b) shown the triploid chromosome pictures for 600µM concentration treatment.

Mean triploidy rate of eggs that had been shocked with three concentrations of 6-DMAP was shown in Table 2. The highest percentage of triploidy rate (100%±0.00) achieved at 200µM of 6-DMAP for 20 minutes. The concentration of 400µM did produce highest triploidy rate (97.8%±3.87) at the duration of 10 minutes and 600µM afforded highest mean triploidy rate (98.9%±1.91) with the durations of 10 minutes. Concentration 600µM resulted highest triploidy rate (75.6%±5.10) for 20 minutes. There was a significant different($P<0.05$) in mean triploidy rates between the groups of different concentrations and the time duration ($P<0.05$).

Table 1: Number of chromosomes counts induced with 6-DMAP

Concentration (µM)	Duration (min)	Number of Chromosomes Mean ± SD
Control	Control	88± 0.00
		88± 0.00
		88± 0.00
200	10	109± 20.00
	15	110± 19.00
	20	118± 12.00
400	10	118± 14.80
	15	113± 19.80
	20	114± 25.40
600	10	108± 18.90
	15	107± 21.50
	20	113± 24.00

Note: 90 egg samples per treatment used (n = 90)

Table 2: Mean triploidy rate of at different concentrations of 6-DMAP. The triploid number of chromosome is range between 89 to 132 numbers of chromosome while diploid number of chromosome contain 88 number of chromosome.

Treatment (µM)	Duration (min)	Number of Diploid	Number of Triploid	Mean Triploidy Rate (%)
Control	Control	90	0	0.00 ± 0.00
		90	0	0.00 ± 0.00
		90	0	0.00 ± 0.00
200	10	2	88	97.8 ± 3.87
	15	2	88	97.8 ± 3.87
	20	0	90	100 ± 0.00
400	10	2	88	97.8 ± 3.87
	15	7	83	92.2 ± 1.91
	20	17	73	81.1 ± 6.97
600	10	1	89	98.9 ± 1.91
	15	11	79	87.8 ± 1.91
	20	22	68	75.6 ± 5.10

Note: 90 egg samples per treatment used (n = 90)

Table 3: Mean hatching rate of triploid induced eggs at different concentrations of 6-DMAP.

Treatment (µM)	Duration (mins)	Mean hatching Rate (%)
Control	Control	78.13±13.75
		74.58±8.14
		85.00±8.21
200	10	85.1 ± 11.1
	15	44.4 ± 3.39
	20	55.0 ± 6.10
400	10	32.8 ± 4.91
	15	22.3 ± 1.10
	20	14.5 ± 0.80
600	10	28.9 ± 1.50
	15	6.2 ± 0.40
	20	3.5 ± 0.50

Note: 3 replicates per treatment used (n = 3)

Hatching Rate: The mean hatching rate of triploid induced eggs was represented in Table 3. Hatching rate was found high in control treatment compared to other treatments. For the treatments, the highest mean hatching rate (85.1%±11.1) was obtained with shock of 200µM of 6-DMAP for 10 minutes. Lowest hatching rate (3.5 ± 0.50) was achieved with 600µM of 6-DMAP for 20 minutes. Different concentrations with different shock period did show different hatching rate. There were significant differences in hatching rate between the treated groups as well as for the duration of shock ($P<0.05$).

DISCUSSIONS

The application of set manipulation of chromosome is one form of bio technology method used to produce quality sterile fish seed for aquaculture activities. In this study, the blocking of the second polar body was done to induce triploidy in *F. Merguiensis* using various concentration of 6-DMAP and time durations. The timing to block the release of second polar body is crucial for an efficient production of triploid. Other factors that would affect the level of triploid in shrimp also include spawning duration and spawning process [18, 19]. The timing, duration of treatment and the toxicity of the treatments are important and may be the reason that no triploids were produced [20]. In a study on Kuruma shrimp, *Marsupenaeus japonicus*, 6-DMAP treatment effectively prevent the release of both first and second polar bodies [21]. The second polar body of white pacific shrimp, *Litopenaeus vannamei* was completely being released at 16 minutes after spawning at the temperature of 28°C and salinity about 33ppt [6]. Therefore, such condition can be used on *F. merguiensis* species.

In the present study the triploid rate was inconsistent not related with duration of time, for example, highest mean triploid rate was achieved at low concentration 200 µM of 6-DMAP at 20 minutes and on other hand, lowest mean triploid rate was achieved at time duration 20 minutes with 600 µM of 6-DMAP concentration. From the result, a relatively high hatching rate was observed at lower concentration of 6-DMAP, however, the rate of hatching of triploid decreases with durations and higher concentration of 6-DMAP. In the study done by [19] also explained a high triploidy rate with low hatching rate in *P. japonicus* when the eggs were shocked in 6-DMAP. Results of present study suggests that timing duration and concentration of 6-DMAP were correlated in term of hatching rate only. Several factors affect the hatching rate. Differences in handling such as collection of eggs and placed in the specially constructed bucket may stress the eggs and also rinsing of the eggs as well as the variation in water temperatures during the treatment process may cause the variation in hatching rate in triploid individuals [21]. The increase in the concentration of 6-DMAP increases its toxicity which may also affect the hatching rate of the triploid eggs. A higher concentration of 6-DMAP may disrupt the metaphase spindles too quickly cause fewer eggs that are able to complete metaphase-anaphase transition during treatment than at lower concentration which result in lower hatching success in triploid induced individuals [22]. This may explain the low hatching rate of triploid eggs at higher concentration. [22] also suggested that a shorter duration of shock at higher concentration may give a better hatching success in triploid induced individuals. Similar finding were determined in this study, lowest concentration of 6-DMAP did produce higher hatching rate.

It has been reported that thermal and pressure shock inhibited the formation of microfilaments and microtubules consequently stop not only the movement of chromosomes, but also cell division and completely block cell development which limit their effectiveness to induce triploidy [17 ; 2]. Moreover, it was reported that triploidy induction by chemical shock particularly 6-DMAP had successfully produce a high triploidy level in Kuruma shrimp, *Marsupenaeus japonicus* [7, 16] and higher triploidy percentage in bivalves [5, 15, 23, 24]. Almost 100% of triploidy were reported in a study of triploidy inducing in pacific oyster, *Crassostrea gigas* using 6-DMAP [13, 23] reported to have successfully induced high percentage of triploidy in geoduck clam, *Panopea abrupt* using 6-DMAP in comparison with the shock using cytochalasin B. The induction of triploidy of abalone, *Haliotis asinina* also showed a great success using 6-DMAP with the highest triploidy level 96.4% at low concentration of 6-DMAP which was at 250µM [20]. [22] Reported a high level of triploidy in blacklip abalone, *Haliotis rubra* (Leach, 1814) at lower concentration of 6-DMAP. There had been a study that reported a successful triploidy induction with high triploidy rate with chemical shock in the Chinese mitten-handed crab, *Eriocheir sinensis* which used cytochalasin B [2]. Therefore, this study explained that chemical treatment is more effective in inducing polyploidy compared than thermal and pressure shock.

During the present study, mosaic individuals (presence of both diploid and triploid in the cells of the same individual) were present in the treated eggs.

Aneuploidy (abnormal number of chromosomes) which causes extra or less number of chromosomes in triploid individuals were also observed in the treated eggs in this study. In general, aneuploidy causes imbalances in gene dosage and often has severe consequences [25]. [18;19] explained the possibility of aneuploidy in shrimp affected the hatching of the triploid eggs because the shrimp could not tolerate aneuploidy caused the eggs failed to hatch. The mosaicism occurs may be due to incomplete inhibition of mitosis in the cells that have two or more nuclei [15]. The occurrence of mosaic offsprings is frequently observed in studies regarding triploidy induction [26]. It was also been reported that mosaics are caused by inappropriate shock treatment when there were inadequate treatment exposure is applied for duplication of chromosomes [27]. Therefore, the shock treatment to banana shrimp, *F. Merguiensis* 6-DMAP at a certain concentration produced triploid offspring.

CONCLUSION

The study showed the effectiveness of 6-DMAP in inducing triploid in *F. merguiensis* had produced relatively high rate of triploid individuals in all treatments ranging from 76% to 100%. Despite the high triploidy rate, the concentration and duration of treatment appeared to have affected the hatching rate of the triploid eggs. The hatching rate appeared to be lower at higher concentration of 6-DMAP and at longer duration. Therefore, further research efforts will need to look at ways to improve triploid treatment inducing with 6-DMAP in *F. merguiensis* to optimize the triploidy level.

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