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# Performance of the First Generation Crossbred Common Carp Bearing Growth and Disease KHV Resistance Related Molecular Markers

Rina[a], Odang Carman[b*], Alimuddin[c], Komar Sumantadinata[d], Muhammad Zairin Jr [e]

[a] *Doctoral Program, Aquaculture Study Program, Bogor Agricultural University (IPB), Indonesia. Agency for Marine Affairs and Fisheries HRD, Ministry of Marine Affairs and Fisheries, Republic of Indonesia. Jln. Medan MerdekaTimur No. 16, Jakarta Pusat 10110.*

[b,c,d,e] *Department of Aquaculture, Faculty of Marine and Fisheries Science, Bogor Agricultural University (IPB), Indonesia*

[a]*E-mail: rinajanwar@yahoo.com*

[c]*E-mail:genetic@indo.net.id*

**Abstract**

Genetic improvement by using marker assisted selection (MAS) can save time and facilities. Common carp (*Cyprinuscarpio* L) in Indonesia has been reported to have DNA marker Cca-08 which related to fast growth, and Cyca-DAB1*05 associated with resistance to infection of koi herpesvirus (KHV). This research was conducted to find out the performance of offspring ten crossbreeding common carp by using the two DNA markers. Broodstocks were collected from Wanayasa Freshwater Fish Seed Development Center and Sukabumi Main Center of Freshwater Aquaculture Development. The existence of Cca-08 marker was analyzed by using microsatellite, while Cyca-DAB1*05 by PCR with specific primer. The results showed that most of broodstock from Wanayasa bearing Cca-08 marker (92.4%), while from Sukabumi was Cyca-DAB1*05 marker (94.4%). Percentage of broodstocks bearing the two markers was 9.4% from Wanayasa, and 38.9% from Sukabumi. Inheritance pattern of the markers in progenies was different among crossbred. All crosses showed 65% or more progenies bear the Cca-08, while the Cyca-DAB1*05 was present with the highest percentage in crossbreeding between female homozygote Cca-08 with Cyca-DAB1*05 and male without Cca-08 and Cyca-DAB1*05

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* Corresponding author.
E-mail address: genetic@indo.net.id

(70%). The best performance of growth and survival rate was obtained in crossbreeding between female heterozygote Cca-08 without Cyca-DAB1*05 and male heterozygote Cca-08 with Cyca-DAB1*05, and this broodstock could be used to produce superior common carp seed.

***Keywords:*** growth; *Cyprinuscarpio*L; resistance to KHV; molecular marker.

**1. Introduction**

Common carp, *Cyprinuscarpio* L, is widely cultured in Asia, Central and Eastern Europe [1]. Aquaculture practices may accidentally decrease the genetic variability in farm by breeding among related individuals or by the use of limited numbers of broodstock. Decreasing genetic quality due to loss of alleles by hatchery activity could influence growth rate, diseases resistance and environmental changes [2,3].

Common carp production in Indonesia has faced some critical problems, such as quality of broodstock, koi hervesvirus (KHV) outbreak, and high price of commercial feed [4]. Selection can provide high quality broodstock and seed to accelerate production. The result of selection is the selected broodstock that will inherit their superiority to derivatives [5].

Application of selection with molecular markers (marker assisted selection/MAS) can accelerate the selection process to get the desired character [6]. The advantages using DNA markers in breeding programs allow to identify prospective broodstock that has a breeding value in early stage. This will reduce the cost for facilities and maintenance large number of fish for progeny tests [7], so the selection using MAS can save time and facilities.

Usage of molecular markers in aquaculture researches have been widely performed such as RAPD with Oki206 marker in association with the flesh color of salmon [8]. Sun and Liang use 5NI451c, 10C900c, 10C1300c and 10C1200 markers to get low temperature tolerant common carp[9]. Backcross of rainbow trout and steelhead trout were used to identify associations between molecular markers determining resistance to infectious of necrotic hematopoietic virus (IHNV) [10]. Microsatellite DNA basically is part of non-genes DNA, but because of the linkage between microsatelliteDNA with genes that control economically traits, the microsatellite DNA becomes effective in selecting economical traits for cattle [11]. Microsatellite DNA markers are useful as a first step in determining desire fish for MASprograms, and can be used to identify the parents of individual, provided both the candidate and its parents [12].

In Indonesia, it has been reported that Cca-08 marker potentially associated with fast growth in common carp [13] and Cyca-DAB1*05 marker associated with resistance of common carp to infection of KHV [14]. Rakus[15] used Cyca-DAB1*05 marker on European common carp which resistant to KHV. Growth performance and resistance to KHV in common carp that associated with their molecular markers are needed to clarify.

## 2. Materials and methods

Research was conducted from May 2012 until February 2014. Identification of broodstock bearing fast growth and resistance to KHV markers was performed at Genetic and Reproduction Laboratory, Aquaculture Department, Faculty of Marine and Fisheries Science, Bogor Agricultural University (IPB), Sukamandi Research Institute for Fish Breeding and Sukabumi Main Centre of Freshwater Aquaculture Development. Fish rearing was conducted at Wanayasa Freshwater Fish Seed Development Centre

### *2.1 Selection for broodstock bearing fast growth and resistance to KHV markers*

Fifty three broodstock from Wanayasa and 18 broodstock from Sukabumi were analyzed. Broodstok from Wanayasa consist of three different populations as a part of growth promotion selective breeding program; which are (1) Founder (P), (2) First generation (F1) and (3) Second generation (F2), while the broodstock from Sukabumi were first generation (F1) of KHV resistance selective breeding program. These broodstock were tagged by microchip and 10 mg of fin tissues were taken then fixed in 90% alcohol. DNA genomic was extracted by DNA isolation kit (Qiagen) according to manual. DNA genomic were then dissolved in 50 µL *ion exchange water*, and stored at -20$^{o}$C until used. Next step was PCR amplification using 5'-GGCTGTTTTACCTCTGTGAA-3' forward and 5'-AAATAACTTTGGACTGCT-3' reverse primers for Cca-08 marker [16], and 5'-CTAATGGATACTACTGG-3' forward and 5'-ATCGCTGACTGTCTGTT-3' reverse primers for CycaDAB-1*05 marker [14].

### *2.2. Cca-08 and CycaDAB1*05 amplification*

Protocol for Cca-08 amplification was performed according to Maskur [13]. Separation of PCR products were conducted by mastermix type it microsatellite(Qiagen) with QIAxcel. The Cca-08 genotype was interpreted based on Chistiakov [17]. Protocol for CycaDAB1*05 amplification, separation PCR products and bands visualization were carried out according to Alimuddin [14].

### *2.3. Crossbred verified broodstock bearing fast growth and resistance to KHV markers*

Ten combinations of crossbred based on the existence of the selected markers (Table1) were done with the following steps: males and females were kept in separate running water ponds. During the process of gonads maturation, the fish were fed on commercial feed (28% crude protein) three times a day. To induced controlled ovulation, the fish were injected by ovaprim (commercial GnRH plus antidopamin mixed) at dose 0.25 ml/kg fish. Eggs and sperms were collected by stripping and fertilization was conducted artificially. Fertilized eggs were then hatched in a 150x80x50 cm fiber glass tank for every corossbred and three-day-old larvae were fed on *Artemia*naupli. After ten days all larvae were moved to 2x2x1 m happa that placed randomly in 27x18x1 m pond at density of 350 larvae per happa. During the first 30 days culture period in happa, the fish were fed *ad libitum* on commercial feed (Hi-provit, 38% crude protein) twice a day. On day 30 tissue sampling were performed to evaluate the offspring bearing markers.

Table 1. Parent stock crossbred combination

| Crossbred (female x male) | Female | | Male | |
|---|---|---|---|---|
| | Cca-08 | Cyca-DAB1*05 | Cca-08 | Cyca-DAB1*05 |
| $M^+xT^+$ | homozygote | + | heterozygote | + |
| $M^+x0^-$ | homozygote | + | - | - |
| $M^+xM^-$ | homozygote | + | homozygote | - |
| $M^+xT^-$ | homozygote | + | heterozygote | - |
| $M^-xT^+$ | homozygote | - | heterozygote | + |
| $M^-x0^-$ | homozygote | - | - | - |
| $M^-xM^-$ | homozygote | - | homozygote | - |
| $T^-xT^+$ | heterozygote | - | heterozygote | + |
| $T^-x0^-$ | heterozygote | - | - | - |
| $T^-xM^-$ | heterozygote | - | homozygote | - |

M = Parent bearing homozygote Cca-08 marker. T = Parent bearing 08 heterozygote Cca-8 marker. (0) = Parent without Cca-08 marker. (+) = Parent bearing Cyca-DAB1*05 marker. (-) = Parent without Cyca-DAB1*05 marker.

Phenotype performances of offspring were evaluated every 15 days by sampling 30 larvae per each offspring up to 90 days rearing period. Data of average weight, growth, survival rate and final biomass were used to evaluate the phenotype performance of offspring linked with existence of markers. Growth rate (%) were calculated according to Huisman [18]. The experimental design used completely random design with three replications.

## 3. Results

### *3.1. Existence of Cca-08 and CycaDAB1*05 in markers verified broodstock*

Evaluation of existence the broodstock bearing both Cca-08 and CycaDAB1*05 markers showed different 5 (five) genotypes; Cca-08 homozygote with CycaDAB1*05 (M+), Cca-08 homozygote without CycaDAB1*05 (M-), Cca-08 heterozygote with CycaDAB1*05 (T+), Cca-08 heterozygote without CycaDAB1*05 (T-), and without both marker (0-). Furthermore majority of the broodstock from Wanayasa have Cca-08 marker (92.4%) andonly 3 females and 2 males (9.4%) bearing Cyca-DAB1*05 marker, while broodstock from Sukabumi bearing 94.4% CycaDAB1*05 marker with only 38.8% broodstock bearing Cca-08 marker.

### *3.2. Cca-08 and CycaDAB1*05 inheritance*

The existence of Cca-08 and Cyca-DAB1*05 markers in each offspring are shown in Table 2, while Cca-08 genotype are presented in Table 3. Results showed that the fast growth related marker (Cca-08) and resistance to KHV marker (CycaDAB1*05) segregated from parent to their derivatives with different frequencies. Crossbred between heterozygote parents resulted more genotype variation (Table 3).

Table 2. Frequency of Cca-08 and Cyca-DAB1*05 markers in offspring

| Crossbred (female x male) | Marker frequency in offspring | |
|---|---|---|
| | Cca-08 (%) | Cyca-DAB 1*05 (%) |
| $M^+ \times T^+$ | 65 | 5 |
| $M^+ \times 0^-$ | 75 | 70 |
| $M^+ \times M^-$ | 100 | 35 |
| $M^+ \times T^-$ | 100 | 45 |
| $M^- \times T^+$ | 100 | 40 |
| $M^- \times 0^-$ | 100 | 0 |
| $M^- \times M^-$ | 95 | 0 |
| $T^- \times T^+$ | 95 | 65 |
| $T^- \times 0^-$ | 90 | 0 |
| $T^- \times M^-$ | 75 | 0 |

M = Parent bearing homozygote Cca-08 marker. T = Parent bearing 08 heterozygote Cca-8 marker. (0) = Parent without Cca-08 marker. (+) = Parent bearing Cyca-DAB1*05 marker. (-) = Parent without Cyca-DAB1*05 marker.

Table 3. Genotype offspring from 10 crossbred combination

| Female genotype | Male: genotype | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | T: 243/276 | 0: -/- | M :270/270 | T :238/270 | T: 254/269 | 0: -/- | M: 234/234 | T:254/269 | 0: -/- | M: 240/245 |
| M- 247/247 | 243/247, (25) | | | | | | | | | |
| | 247/276 (40) | | | | | | | | | |
| M: 244/244 | | 244/244 (75) | | | | | | | | |
| M: 234/234 | | | 234/270 (100) | | | | | | | |
| M: 245/245 | | | | 238/245 (75) | | | | | | |
| | | | | 245/270 (25) | | | | | | |
| M: 238/238 | | | | | 238/254 (40) | | | | | |
| | | | | | 238/269 (60) | | | | | |
| M- 238/238 | | | | | | 238/238 (100) | | | | |
| M- 238/238 | | | | | | | 234/238 (95) | | | |
| T: 245/251 | | | | | | | | 245/254 (20) | | |
| | | | | | | | | 245/269 (25) | | |
| | | | | | | | | 251/254 (15) | | |
| | | | | | | | | 251/269 (35) | | |
| T: 245/251 | | | | | | | | | 245/245 (90) | |
| T- 245/251 | | | | | | | | | | 240/245 (30) |
| | | | | | | | | | | 240/251 (45) |

M = Parent bearing homozygote Cca-08. T = Parent bearing heterozygote Cca-08. 0 = Parent without Cca-08. Value in parenthesis ( ): frequency.

### *3.3. Phenotype performances of offspring*

Growth performances of offspring from each crossbred during 90 days rearing period are presented in Figure 1. Survival rate, biomass after 90 days rearing period and daily growth rate are shown in Table 4. The highest value for all parameters were obtained from crossing between Cca-08 heterozygote female without CycaDAB1*05 and Cca-08 heterozygote male with CycaDAB1*05 ($T^- xT^+$).

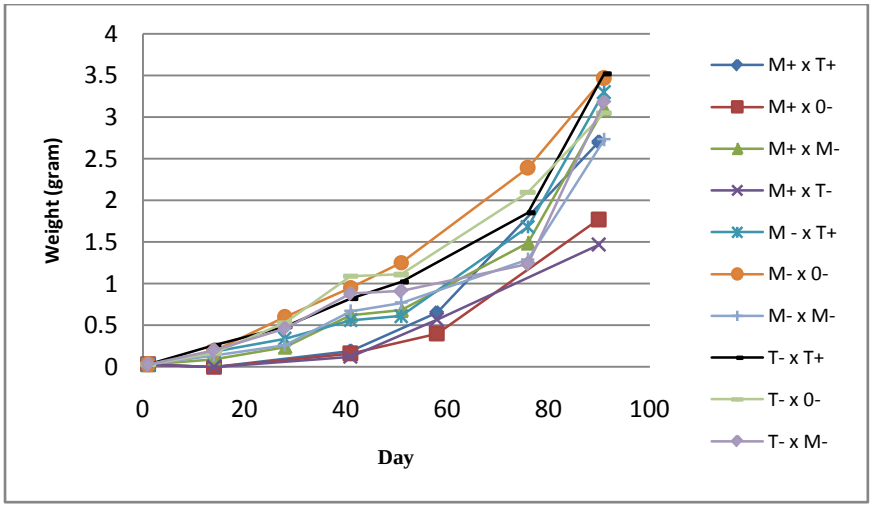


Figure 1.Growth of offspring during 90 days rearing period.MxT= M female cross with T male. M= Parent bearing homozygote Cca-08 marker. T = Parent bearing 08 heterozygote Cca-8 marker. (0) = Parent without Cca-08 marker. (+) = Parent bearing Cyca-DAB1*05 marker. (-) = Parent without Cyca-DAB1*05 marker.

Table 4. Survival rate, biomass and the daily growth rate for offspring

| Crossbred (female x male) | Survival rate (%) | Biomass (gr) | Daily growth rate (%) |
|---|---|---|---|
| $M^+ \times T^+$ | 19.7 | 186.3 | 4.9±0.30 |
| $M^+ \times 0^-$ | 49.1 | 304.4 | 4.31±0.70 |
| $M^+ \times M^-$ | 35.7 | 387.4 | 5.19±0.36 |
| $M^+ \times T^-$ | 36.6 | 188.16 | 4.22±0.14 |
| $M^- \times T^+$ | 55.4 | 640.2 | 5.23±0.49 |
| $M^- \times 0^-$ | 29.7 | 413.92 | 5.29±0.49 |
| $M^- \times M^-$ | 36.0 | 343.98 | 5.07±0.19 |
| $T^- \times T^+$ | 76.9 | 863.49 | 5.37±0.09 |
| $T^- \times 0^-$ | 46.6 | 497.15 | 5.17±0.26 |
| $T^- \times M^-$ | 51.4 | 527.4 | 5.18±0.52 |

M= Parent bearing homozygote Cca-08 marker. T = Parent bearing 08 heterozygote Cca-8 marker. (0) = Parent without Cca-08 marker. (+) = Parent bearing Cyca-DAB1*05 marker. (-) = Parent without Cyca-DAB1*05 marker.

### *3.4. Discussion*

Based on the results of the analysis of the existence of fast growth and resistance to KHV markers in broodstock, revealed that the majority broodstock bearing Cca-08 in Wanayasa and broodstock bearingCycaDAB1*05 in Sukabumi are in line with the goal of each selective breeding program. The existence of the Cca-08 marker was detected and clearly segregated in its derivatives, but not all offspring bearing Cca-08 marker. Microsatellite is codominant, and is inherited Mendelian [19, 20]. In this research there is not enough data to explain the Mendelian pattern due to the sampling method (no census) as well as the recording of dead fish during rearing period (not analyzed). Based on genotypes for 10 crossbred combination, crossbred between parents bearing heterozygote Cca-08 provide more genotype variability compared to the crossbred between parents bearing homozygote Cca-08. In general, increasing the percentage of heterozygote will increase the vitality of livestock populations.

Similar with the fast growth marker, the resistance to KHV marker (CycaDAB1*05) was also inherited and segregated in their derivatives. TheCyca-DAB genes are segregated independently [21]. Inheritance of CycaDAB1*05 marker that seemly doesn't Mendelism due to the similar reason as mention above. Crossbred between parents do not bearing CycaDAB1*05 marker do clearly produced offspring without this marker. The low percentage of CycaDAB1*05 maker in crossbred between females bearing homozygote Cca-08 without Cyca-DAB1*5 markers and male bearing heterozygote Cca-08 and Cyca-DAB1*05 markers is assumed due to the low viability of the offspring. This condition was correlated with their survival rate.

Statistical analysis of 10 crossbred biomass indicated significantly different between $T^- xT^+$ with $M^+ xT^+$, $M^+ x\ 0^-$, $M^+ x\ M^-$, $M^+ x\ T^-$, $M^- x0^-$ $M^- xM^-$, $T^- x0^-$ and $M^- xT^+$ but similar with $T^- xM^-$. Generally crossing females bearing heterozygote Cca-08 tend to provide higher growth rate offspring compared with the crossing females bearing homozygote Cca-08. Based on daily growth rate during 90 day of rearing, showed the highest value (5.37%) was obtain in $T^- xT^+$ crossing. Significant growth rate in these crossbred is matched with the high frequency (95%) of offspring bearing Cca-08 marker.

The presence of fast growth and resistance to KHV markers in 10 crossbred combinations has a strong correlation with the phenotype performance. Crossbred between females bearing heterozygote Cca-08 with male bearing heterozygote Cca-08 and CycaDAB1*05 marker ($T^- x\ T^+$) tend to give the best result in term of survival rate and growth. On the other hand, crossbred between a female bearing both homozygote Cca-08 and Cyca-DAB1*05 with male bearing both heterozygote Cca-08 and Cyca-DAB1*05 ($M^+ x\ T^+$) tend to give the lowest biomass and survival rate.

**4. Conclusions**

Fast growth and resistance to KHV markers on common carp was inherited to offspring with different frequencies. Crossbred between females bearing heterozygote fast growth without resistance to KHV marker with male bearing heterozygote fast growth with resistance to KHV markers provide the best performance in term of survival rate and growth.

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