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Assessment Selective Breeding Effect of Israeli carp (*Cyprinus carpio*) from Korea

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ABSTRACT Since the introduction of Israeli carp into Korea for farming in 1973, there are no breeding studies on developing Korea Israeli carp (domestic) so far. This study performed gene-based cross-breeding studies to restore genetic diversity of lowered Israeli carp through continuous inbreeding, and for rapid growth and better scales. This study produced four cross-breeding groups (F1) using Koean Israeli carp and Chinese Songpu mirror carp for the improvement of growth and scale of Israeli carp in Korea. And mating scheme for breeding groups was set in consideration of the morphological analysis and genetic distance of broodstock. In addition, this study used microsatellite markers and genotype data to analyze genetic diversity and parentage analysis. As a result, the average N_A and H_E values of Korean select broodstock are 8.3 and 0.743, and F1 is 13.0 and 0.764. This study shows that the genetic diversity of F1 has been recovered over Korean Israeli carp through breeding between Korean Israeli carp and Chinese Songpu mirror carp. Common Israeli carp in Korea reached 1.7 kg in 17 months, and improved Israeli carp reached to 2.2 kg. The KC (Korea × China, KC) group was 2.52 and broodstock group was 3.15. F1 showed lower scale score (0.63) than broodstock. The improved carp (F1; CK, KC) had 20% better scales than the parent group (F0), which improved 27% in weight and 25% in scales compared to common Israeli carp. The Israeli carp developed by the genetics-based breeding grew quicker and had improved genetic diversity and fewer scales, which will be of great value for Korean Israeli aquaculture industry due to good marketability.

Key words: Israeli carp, *Cyprinus carpio*, genetic breeding, microsatellite markers, parentage analysis, scales

INTRODUCTION

Common carp is one of the species that make up the aquaculture industry in different countries in Europe and Asia. Common carp breeding has been studied in many countries worldwide (Moav and Wohlfarth, 1976). The improved carp developed by breeding is then exported to other countries. In particular, carp breeding studies brought early prosperity in Europe (Tal and Sheluvki, 1952; Yashouv, 1955; Hulak *et al.*, 2010). The best breed of the Israeli carp had been established in the 1970s, called "Dor 70 strain" or "Israeli mirror carp". During this time, South Korea and China imported carp from Israel (Wohlfarth *et al.*, 1980; Sin, 1982). The Israeli carp strain is currently the most widely distributed fish in the world and is being cultured in many parts of Europe and Asia (Wohlfarth *et al.*, 1983; Li *et al.*, 2007).

Since the introduction of Israeli carp into Korea for farming in 1973, inland fish-aquaculture flourished dramatically because Israeli carp became the new popular dish among Korean people. Wide use of net cage in reservoirs made mass production of freshwater fish possible (Kim, 2016; Kim, 2018). However, the popularity of this fish for human consumption also dropped dramatically since 1998 when the carp stocks were affected by a variety of diseases and parasites. More than 70% of carp farms in many regions of the country have suffered from heavy economic losses due to disease outbreaks (Oh *et al.*, 2001), and some Israeli

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carp aquafarms were forced to close in Korea. Mass mortality during the events was caused by the low immunity of the highly inbred fish stocks. The same problem is known to have occurred among carps in Bangladesh (Rajts *et al*., 2002; Mondol *et al*., 2006). In addition, loss of genetic diversity using the inappropriate mating scheme is blamed for the mass mortality. Simple general mass mating was carried out in the fish farms in Korea without any designed approaches or a mating scheme for selective breeding. This approach is believed to have caused a rapid decline of genetic diversity as only some groups of fish were repeatedly involved in mating. It is urgent to study breeding improvements considering the genetic diversity and distance of Israeli carp in Korea (Kim, 2018).

Fish breeding is an important global issue, and many previous reports related to a variety of fish including Atlantic salmon, tilapia, common carp and rainbow trout are available. For example, Norway had notable success in government-operated research on Atlantic salmon breeding in 1968 (Gjedrem, 2012). Norway captured 90% of the global salmon market, and its tilapia market grew by 20% each generation. China has developed and commercialized a superior breed of common carp by integrating traditional method for breeding with molecular breeding technique (Dong *et al*., 2015). German mirror carp (*Cyprinus carpio*) is a popular freshwater fish reared in Europe because of its fast growth rate, feeding efficiency, cold resistance, and ease for domestication. The German mirror carp was introduced to China in 1984 but could not adapt well to foreign aquaculture conditions (Li *et al*., 2009). Therefore, the Heilongjiang River Fishery Institute conducted a breeding study for obtaining an improved variety (Li *et al*., 2009). After several years, a selected strain called the "Songpu mirror carp" was successfully bred. It has the positive characteristics of the German mirror carp, such as few scales and high growth, high survival rate, and improved disease resistance (Li *et al*., 2009).

Korean fishermen prefer the type of Israeli carp, which have fewer scales and higher the body height. Because fewer scales and height body height are easier to fish trimming and get more the edible portion weight so therefore more in demand commercial. In addition, the size and shape of the Israeli carp in Korea are currently undesirable. However, no breeding studies have been conducted since the introduction of Israeli carp in the 1970s to develop a superior breed in Korea (Kim, 2018). Since the products of inland-water fish farming is an important part of national food security, developing an improved fish breed with a strong potential for commercially competitive value is crucial in sustaining national economy (Korea Fisheries News, 2015). In order to improve the breeding of Korean Israeli carp, a previous study was conducted to improve the size and scale by crossing with Chinese species (Hwang *et al*., 2016).

The purpose of this study was to develop an Israeli carp breed that grows fast and has improved body shape and scale by using genetic breeding methods. In this study, we produced the F1 generation according to a planned mating scheme. Subsequently, a parentage test, genetic diversity evaluation, and morphological evaluation of the F1 group were performed to selected an improved broodstock.

MATERIALS AND METHODS

1. Sample collection

Chinese Israeli carp was imported from Chinese Academy of Fishery Sciences, Heilongjiang River Fisheries Institute for research purposes. This study was carried out under the permission (2017-NIFS-IACUC-13) from the National Institute of Fisheries Science, NIFS, South Korea. We declare that all of the experiments described herein comply with the current laws of Korea (Ordinance of Agriculture, Food and Fisheries, No. 1, Regarding Experimental Animals, no. 9932). Israeli carp (*C. carpio*) fishes were collected from Chungnam (KorA, 47 samples, 2～3-year-old) and Jeonbuk (KorB, 51 samples, 2～3-year-old) in Korea, and samples of cultured Chinese Songpu mirror carp (*C. carpio*) were collected from Jilin province (ChA, 76 samples, 2～3-year-old) and Heilongjiang province (ChB, 80 samples, 2～3-year-old) in China. Fin samples were preserved in 99.9% ethanol and transferred to the laboratory (Table 1).

2. Basic morphological analysis

A total of 98 of Korean Israeli carps and a total of 156 Chinese Songpu mirror carp carps were measured using a ruler and Vernier calipers for total length, body length, weight and body height. Condition factor (CF) and body

Table 1. Country, location, code, and numbers of samples used in this study

Country	Location	Code	No. of sample
China	Jilin	ChA	76
	Heilongjiang	ChB	80
Korea	Chungnam	KorA	47
	Jeonbuk	KorB	51

shape were calculated using the following formula: CF; Body weight (g)/Standard length (mm)^3 × 100,000, Body shape; Body weight (g)/Standard length (mm). Fish were classified into 5 classes based on a 5-step scoring system of scale distribution: 1 score (0∼3 scales), 2 score (4∼10 scales at some dorsal fin scales), 3 score (14∼17 scales at dorsal fin and tail), 4 score (18∼33 scales at dorsal fin, tail and sideline) and 5 score (≥34 scales at dorsal fin, tail and sideline and whole abdomen). RFID (Radio Frequency Identification) tags were inserted into all individuals of the broodstock, and the morphometric analysis each individual was managed by measuring the total length, body length, weight, CF, body shape and scale value according to each unique number (Kim, 2018).

3. Broodstock selection and mating scheme

The breeding experiments of cultivated Israeli carp were carried out using the broodstock F0 carps, which consists of the Korean (domestic) and the Chinese Songpu mirror carp. In the spring, sexually mature breeders were selected for production of families. Selection of broodstock was based on genetic analysis and morphological analysis. Based on genetic analysis of a total of 254 broodstock, male individuals with a calculated value of the genetic relationship with females and a genetic distance value of 0.2 or less were excluded. The genetic distance was computed with the Arlequin software to stablish genetic distances among sample. Genetic distances were estimated between all population pairs based on the chord distance (Cavalli-Sforza and Edwards, 1967). Overall, 100 broodstock samples were selected based on morphological characteristics (Chinese ♀; 19, Chinese ♂; 56, Korean ♀; 11, Korean ♂; 14). Specifically, the total weight, CF, and scale score were converted into 10 points, and the breeding value was calculated by the total weight (70%), CF (20%), and scale score (10%, scale score 2 or less) for each item, i.e., breeding value (BV) = (Weight × 0.7) + (CF × 0.2) + (Scale × 0.1). A total of four mating groups (Chinese C; ♀ × Chinese C; ♂), (Chinese C; ♀ × Korean K; ♂), (Korean K; ♀ × Chinese C; ♂), (Korean K; ♀ × Korean K; ♂) were set up to produce F1. We selected a Korean Israeli carp with mean measurement traits and set up a mating scheme to compare with common Israeli carp in Korea. According to the mating scheme, female and male were placed in each tank and spawned through water temperature stimulation (maintenance at 25°C). When egg spawning completed, eggs were collected and hatched using an artificial incubator. In order to determine the objective breeding efficiency and to utilize the tank efficiently, it was mixed and kept in a single tank for up to 17 months in the same breeding conditions. Fins were collected for genotype analysis of 1,500 surviving (17-month-old) fish. Multiplex PCR was performed using a microsatellite marker, and genetic diversity and parentage analysis were performed using amplified products. Fry was raised in indoor tanks until the early 30 days of age, and then in outdoor tanks. Feeding was 2 times a day, 3∼5% of body weight, and the average water temperature was16 ± 1.0°C, DO was 6.89 ± 2.4 mg/L, pH was 6.50 ± 1.5.

4. Morphological analysis of F1 group

The produced F1 was analyzed from 20 to 170 days after hatching and once more at 17 months. Fish (1,500 samples) were randomly sampled from the tank, and used for growth and scale measurements and genotype analysis. Length and individual mean weight were measured using the ZEN lite 2012 software program. Then, an RFID tag was inserted into each carp of the selected F1 generation offspring, and the metric trait data for each individual were collected by measuring the total length, height, weight, CF, and scale score for each unique RFID number.

5. DNA extraction and microsatellite amplification

Fin samples of F1 (1,500 samples) and broodstock F0 (100 samples) were collected and preserved in 99.9% ethanol and transferred to the laboratory for DNA extraction (Yue and Orban, 2001). Each sample was mixed with 5% Chelex 100 (Bio-Rad Laboratories, Hercules, USA) and 1% proteinase K (Roche, USA) in sterile water, incubated at 55°C for 1 hour and then incubated at 100°C for 10 min. The mixture was centrifuged, and the supernatant was retained. We have chosen markers that had a PIC of 0.5 or higher, it has chosen markers that can accurately track inheritance of alleles, by producing a variety of alleles clearly distinguishable in peak readings. For selected markers, they are clearly distinguished by one or two PCR products inherited from each parent. The six suitable markers were selected to distinguish diversity in carp. For PCR, we used primer pairs designed to amplify markers MFW1, MFW14, MFW16, MFW24, MFW26, and MFW30 (Crooijmans *et al.*, 1997) and subjected the forward primers to fluorescent dye labeling. The following primers were used: MFW01, forward 5′-GTCCAGACTGT CATCAGGAG-3′ and reverse 5′-GAGGTGTACACTG AGTCACGC-3′; MFW14, forward 5′-CAGAAGCTTCT GGAAATCTGAG-3′ and reverse 5′-GCGAGAAGATT GATGGACAAC-3′; MFW16, forward 5′-GTCCATTGT GTCAAGATAGAG-3′ and reverse 5′-TCTTCATTTCAG

GCTGCAAAG-3′; MFW24, forward 5′-GCTCCAGATT GCACATTATAG-3′ and reverse 5′-CTACACACACG CAGAGCCTTTC-3′; MFW26, forward 5′-CCCTGAGA TAGAAACCACTG-3′ and reverse 5′-CACCATGCTTG GATGCAAAAG-3′; MFW30, forward 5′-GGTCAA CAAGTAGTTGTGCAG-3′ and reverse 5′-CCATCTCT GTCATTGCAACAG-3′. Each 20 μL reaction mixture contained 2∼4 μL template DNA (100∼150 ng), forward and reverse primers each 0.5 mM, 1x F-star Taq Reaction buffer (BioFact, Korea), 0.2 mM of dNTP (BioFact, Korea), 1.25 U of F-star Taq DNA polymerase (BioFact, Korea), and 1 μL dimethyl sulfoxide (Sigma St Louis, USA). Thermal cycling was conducted on an Applied Biosystems thermal cycler (#9902) using the conditions as follows: 95°C for 2 min followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. The generated products were confirmed by 2% agarose gel electrophoresis.

6. Analysis of genotype

For fragment analysis, 1 μL of each PCR product was combined with formamide and a 500 LIZ size standard (GeneScan™ 500 LIZ; Applied Biosystems, USA), and the mixture was subjected to capillary electrophoresis on an ABI 3130 DNA Sequencer (Applied Biosystems, USA). The results were analyzed using the GeneMapper version 4.0 fragment analysis software (Applied Biosystems, USA).

7. Statistical analysis

Results were expressed as mean±SD, and SPSS program was used for statistical significance test of all mean values for each measurement value. Significance was tested by t-test and ANOVA. The significance criterion for all P values was 0.05. The data were analyzed by one-way ANOVA using the SPSS statistical package (SPSS 5.0; SPSS Inc., USA). Means were separated using Duncan's multiple range test and were considered significantly different at $P<0.05$ (Duncan, 1955). The numbers of alleles per locus, allele frequencies, expected heterozygosities, and observed heterozygosities were calculated using CERVUS 3.0 (Kalinowski *et al*., 2007). The allele richness of each sample were estimated using FSTAT version 2.9.3 (Goudet, 2001). The parentage test was performed using the PAPA 2.0 program using genotyping data.

RESULTS

1. Broodstock selection and F1 generation production

We analyzed gene distance and morphological characteristics of fish for a genetic breeding. In a previous study, we had analyzed the genetic diversity and distance between Chinese broodstock and Korean broodstock relatives (Kim *et al*., 2018). Genetic analysis showed that the genetic distance between the Korean and the Chinese carps ranged from 0.432 to 0.594 on average, and the genetic distance was at least 0.2 or more. The scale scores were 3.71 (KorA), 3.89 (KorB), 2.38 (ChA) and 2.61 (ChB). In the case of Korean Israeli carp, the scale score was mainly 4, while the scores of Chinese Songpu mirror carps were mainly 1. The average scale index of the Korean carps was 3.8, and the average scale index of the Chinese carps was 2.5, indicating that the Chinese carps had less scales than the Korean carps (Fig. 1, Table 2). In addition, top 40% broodstock was selected via morphological analysis. As a result, 100 out of 254 F0 broodstock were selected. The F1 was produced according to the mating scheme. Specifically, they were selected as 11 Korean females, 14 Korean males, 19 Chinese females, 56 Chinese males. A total of four mating groups were set up and then produced F1 in each tank. Egg production per group was 400 mL for CC, 8,000 mL

Table 2. Morphological characteristics analysis of the Korean and the Chinese Songpu mirror carps (*Cyprinus carpio*)

Country	Code	TL (mm)	BL (mm)	BH (mm)	TW (g)	CF	Body shape	Scale score
Korea	KorA	400.85 ± 24.22^{a}	334.35 ± 19.60^{a}	127.55 ± 10.71^{a}	1286.52 ± 237.60^{a}	1.99 ± 0.32^{b}	0.32 ± 0.02^{b}	3.71 ± 0.49^{b}
	KorB	422.59 ± 35.11^{b}	351.59 ± 30.01^{b}	143.05 ± 13.87^{b}	1713.14 ± 433.73^{a}	2.23 ± 0.23^{c}	0.34 ± 0.02^{c}	3.89 ± 1.27^{b}
	Mean±SD	411.72±29.67	342.97±24.81	135.30±12.29	1499.83±335.67	2.11±0.28	0.33±0.02	3.80±0.88
China	ChA	464.56 ± 31.66^{c}	384.35 ± 29.72^{c}	141.22 ± 7.62^{b}	1708.27 ± 269.84^{b}	1.70 ± 0.18^{a}	0.31 ± 0.02^{a}	2.38 ± 1.10^{a}
	ChB	459.18 ± 30.68^{c}	379.60 ± 27.55^{c}	139.31 ± 8.99^{b}	1681.13 ± 289.38^{b}	1.74 ± 0.20^{a}	0.30 ± 0.02^{a}	2.61 ± 1.49^{a}
	Mean±SD	461.87±31.17	381.98±28.64	140.27±8.31	1694.70±279.61	1.72±0.19	0.31±0.02	2.50±1.30

TL; total length, BL; body length, BH; body height, TW; total weight, CF; condition factor, Body shape; height/length ratio, Values represent mean±standard deviation; means in the same column with different letters are significantly different ($P<0.05$) as determined by Duncan's multiple range test. Values not sharing a common or same alphabet letter (a∼c) and they differ significantly at $P<0.05$.

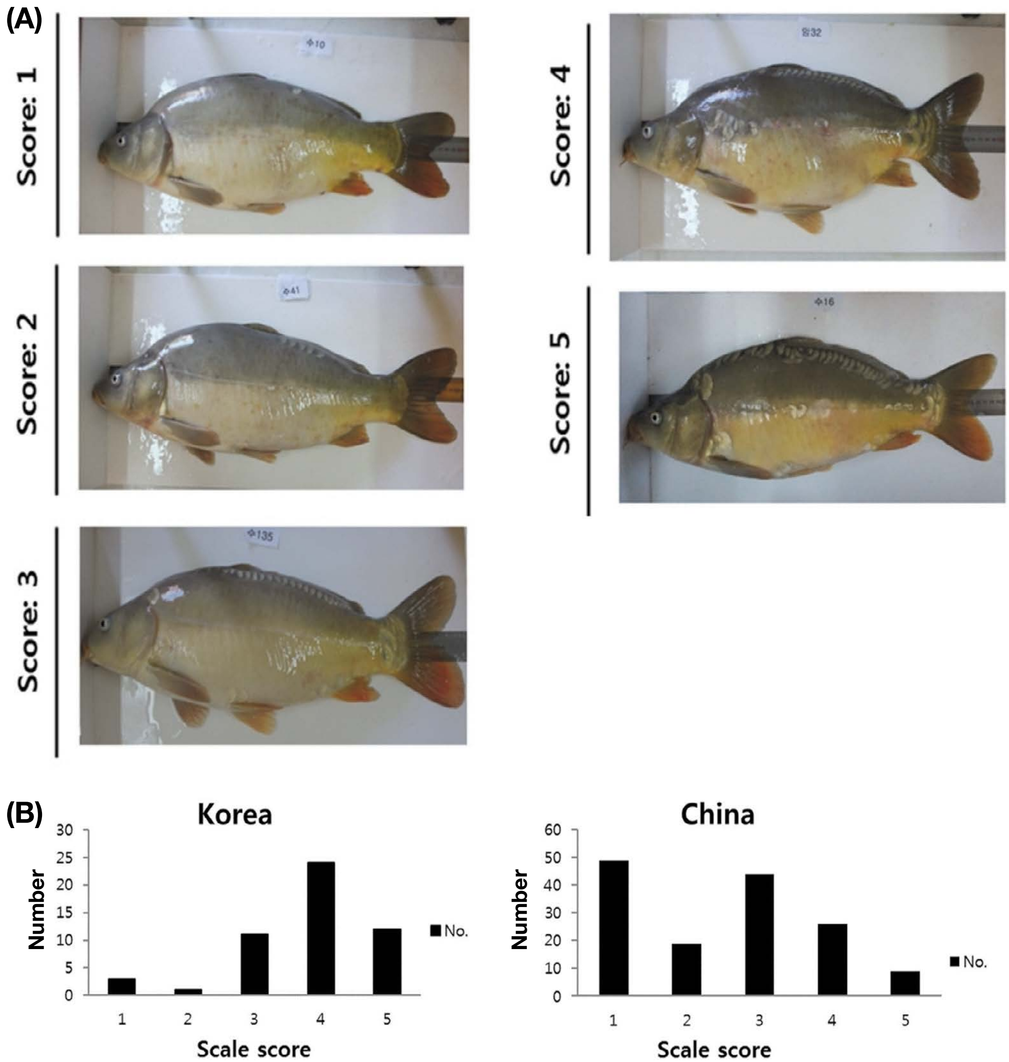

Fig. 1. Evaluation standard set of scale expression in *Cyprinus carpio* broodstock (A). In order to estimate the incidence of scales, the scoring system was set from 1 to 5 according to the degree of a body scale. Scale index measured for each group was based on the score. Distribution of scale scores among the broodstock F0 fishes of the Korean Israeli carps and the Chinese Songpu mirror carps (B).

Table 3. Breeding combination for seed production

Group	Parental Combination	Egg production (mL)
CC	Chinese Songpu mirror carp (♀) × Chinese Songpu mirror carp (♂)	400
CK	Chinese Songpu mirror carp (♀) × Korean Israeli carp (♂)	8,000
KC	Korean Israeli carp (♀) × Chinese Songpu mirror carp (♂)	3,000
KK	Korean Israeli carp (♀) × Korean Israeli carp (♂)	3,000
Common Israeli carp	Korean Israeli carp (♀) × Korean Israeli carp (♂)	1,000

for CK, 3,000 mL for KC and KK, 1,000 mL for common Israeli carp. Hatching was achieved in an artificial incubator (Table 3).

2. Genetic diversity analysis

The genetic diversity of the F1 group produced from the selected F0 broodstock was evaluated using 6 microsatellite markers. A total of 100 (N) individuals in the total brood-

Table 4. Genetic diversity analysis of selected Korea broodstock (F0), selected total broodstock (F0) and F1

Population	Microsatellite loci							
	Parameter	MFW01	MFW14	MFW16	MFW24	MFW26	MFW30	Average
Selected Korea Broodstock (F0)	N_A	8	9	7	10	11	5	8.3
	N	24	24	24	24	24	24	24.0
	PIC	0.754	0.648	0.663	0.659	0.796	0.62	0.690
	H_O	0.875	0.583	0.542	0.917	0.750	0.333	0.667
	H_E	0.800	0.698	0.726	0.705	0.835	0.692	0.743
Selected Total (Korea&China) Broodstock (F0)	N_A	21	13	19	16	20	10	16.5
	N	100	100	100	100	100	100	100.0
	PIC	0.861	0.815	0.864	0.862	0.882	0.688	0.828
	H_O	0.909	0.778	0.717	0.899	0.889	0.414	0.767
	H_E	0.878	0.839	0.880	0.878	0.896	0.728	0.849
Offspring (F1)	N_A	16	10	15	12	16	9	13.0
	N	1253	1253	1253	1253	1253	1243	1251.3
	PIC	0.813	0.686	0.737	0.703	0.841	0.628	0.735
	H_O	0.822	0.802	0.495	0.690	0.935	0.302	0.674
	H_E	0.831	0.718	0.761	0.740	0.855	0.677	0.764

Abbreviation: N_A, number of alleles; N, number of individuals; PIC, polymorphism information content; H_O, observed heterozygosity; H_E, expected heterozygosity.

stocks (Korea + China) were selected, and among them, a total of 24 (N) individuals were selected from Korea, and a total of 1,253 (N) samples in the F1 group were used for diversity analysis (Table 4). In comparison with the results of the diversity analysis of the selected Korean broodstock and F1 generation group, the average number of alleles (N_A) in selected Korea broodstock was 8.3, and the average number of alleles of F1 was 13.0. The average expected heterozygosity (H_E) of selected Korean broodstock was 0.743 and F1 was 0.764, F1 group increased the diversity by 0.021 compared to the selected Korea broodstock (Table 4).

The average number of individuals (N) of selected total broodstock was 100, and the average number of alleles of F1 was 1251.3. The average number of alleles (N_A) of selected total broodstock was 16.50, and the average number of alleles of F1 was 13.0. The average polymorphism information content (PIC) was 0.828 in selected broodstock and 0.735 in offsprings. The observed heterozygosity (H_O) ranged from 0.414 to 0.909 in the selected total broodstock (F0) samples and from 0.302 to 0.935 in the offspring (F1) samples. In contrast, the average observed heterozygosity was high in the selected total broodstock (0.767) than the offspring (F1) (0.674). The expected heterozygosity (H_E) ranged from 0.728 to 0.896 in selected total broodstock and from 0.677 to 0.855 in offspring samples, the average expected heterozygosity was high in the selected total broodstock (0.849) than the offspring (F1) (0.764). On average, the numerical values (N_A, PIC, H_O, H_E) tended to be higher for the selected total broodstock than for the offspring samples. Some unique alleles of the broodstock were considered to be alleles disappeared by bottlenecks over the generation (Fig. 2).

3. Parentage test and family analysis

Parentage analysis of total 1,398 (F1) individuals showed 93% (1,398/1,500) success rate. The parentage analysis was conducted to identify the cross groups of individuals. As a result of parentage analysis, KC (441) was the highest, followed by KK (408), CK (235) and CC (136). The number of families was the highest in KC (60), followed by CC (59), CK (37), KK (36). Common Israeli carp identified 138 individuals and 28 families.

4. Morphological comparison of F1

On the 170th day, the size of the CC, CK, and KC groups was measured at 90.32 ± 22.64 mm, 90.80 ± 16.90 mm, and 88.31 ± 19.00 mm, respectively (Hwang *et al.*, 2016). The size of KK group was measured as 76.77 ± 14.58 mm. The measured size on the 170th day was larger in the CC, CK, and KC groups than that in the KK group.

In this study, we compared and analyzed the morphology characteristics at 17 months. At 17 months of age, the total

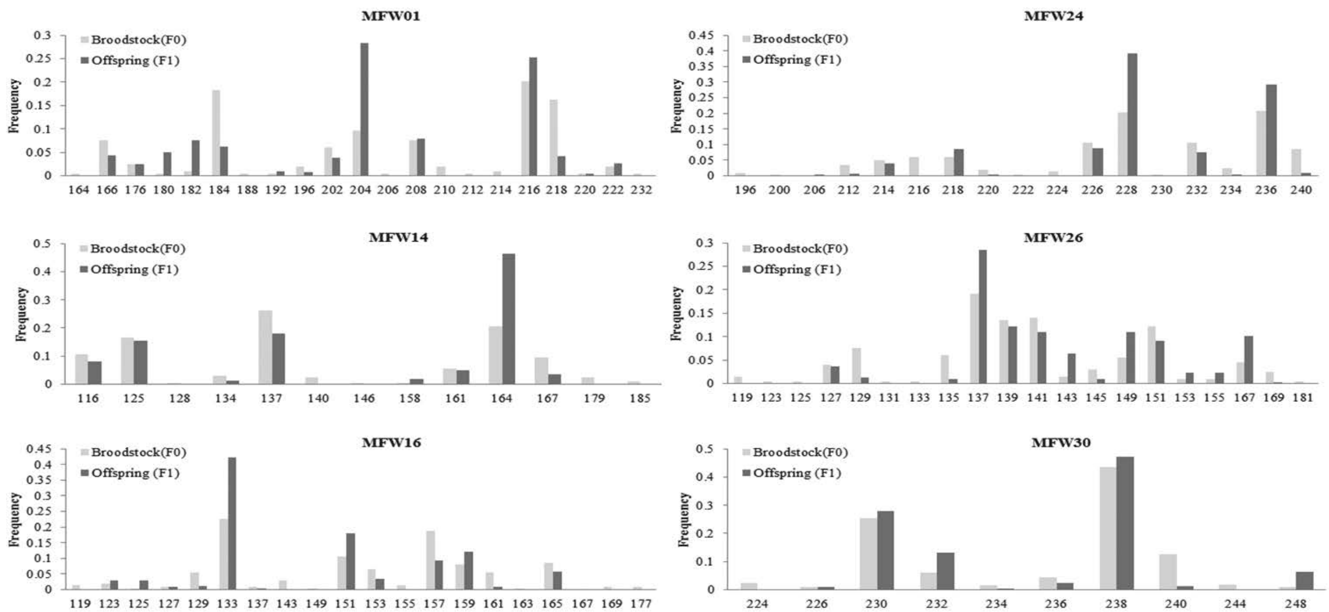

Fig. 2. Allele frequencies in the broodstocks (F0) and F1 group for the 6 microsatellite loci.

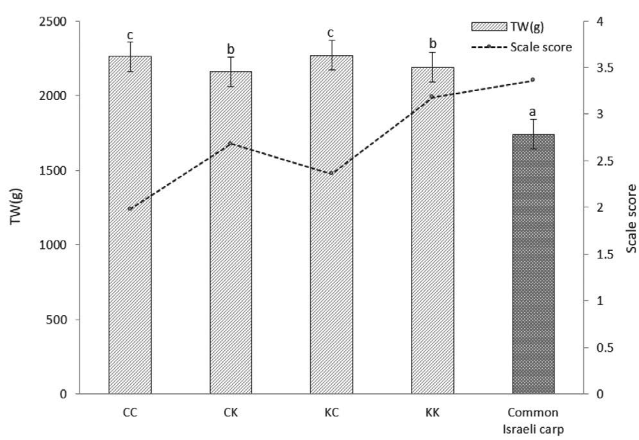

Fig. 3. Comparison of scale and weight in the F1 group and common Israeli carp.

length and weight of four groups were as follows: CC (483.60 ± 25.29 mm, 2260.00 ± 260.00 g), CK (471.52 ± 23.31 mm, 2160.00 ± 240 g), KC (491.77 ± 26.14 mm, 2270.00 ± 260 g) and KK (475.72 ± 23.11 mm, 2190.00 ± 250.00 g) (Fig. 3). Total length and weight were the highest in KC, followed by CC, KK, and CK groups. However, the total length of CK group was the lowest, but body height was similar to that of the other groups. CK was characterized by high body height. CK showed the shortest body length, but its CF value was the highest. Analysis of F1 breeding groups revealed that CC and CK rapidly grew in terms of overall length and body weight since early stages of the life cycle. The participation of Chinese parents was considered to have a positive impact on initial growth. The late growth (17 months) was significantly higher in KC, and it was also found that the height of Korean Israeli carps was high. High growth rate and body shape improvement were observed in Chinese and Korean hybrids. The results excellently exemplified the positive effect of the crossbreeding (Table 5). Besides, the difference in total length between the CK, KC, and the common Israeli carp was 39.80 mm and the weight was 475 g. The common Israeli carp reached 1.7 kg in 17 months, and improved Israeli carp reached 2.2 kg.

5. Distribution of scales in the F1 generation

In the analysis of scale scores of F1 carps, the average scale score was 2.55. The scale scores of the CC group were 1.98 ± 1.15, while it was 2.68 ± 1.05 in the CK group, 2.36 ± 1.34 in the KC group and 3.18 ± 0.82 in the KK group (Fig. 3). The scale score was the lowest in CC group, followed by KC, CK and KC. The scale score of the F1 breeding groups (CK, KC) was 2.52, indicating that F1 breeding groups had fewer scales than broodstock (3.15) (Fig. 3). In particular, it was confirmed that scale distribution was much improved relative to the average of common Israeli carp in Korea (3.36). The scale distribution was less in the mating group, in which more Chinese-born mothers participated than the KK group, and the scale trait of Israeli carp could be effectively managed by cross-breeding.

Table 5. Morphological values of carps by mating group on the 17 month

Age	Morphology	CC	CK	KC	KK	Common Israeli carp	Average
17 month	Sample	136	235	441	408	178	305
	TL (mm)	483.60 ± 25.29^{d}	471.52 ± 23.31^{b}	491.77 ± 26.14^{e}	475.72 ± 23.11^{c}	441.85 ± 22.52^{a}	472.89 ± 24.07
	BH (mm)	156.77 ± 8.48^{b}	156.48 ± 8.54^{b}	154.18 ± 8.08^{b}	156.53 ± 8.15^{b}	139.66 ± 8.55^{a}	152.724 ± 8.36
	TW (g)	2260.00 ± 260^{c}	2160.00 ± 240^{b}	2270.00 ± 260^{c}	2190.00 ± 250^{b}	1740.00 ± 220^{a}	2124.00 ± 246
	CF	2.00 ± 0.22^{b}	2.06 ± 0.23^{c}	1.91 ± 0.23^{a}	2.04 ± 0.24^{b}	2.01 ± 0.25^{b}	2.00 ± 0.23
	Body shape	0.32 ± 0.10^{b}	0.33 ± 0.10^{c}	0.31 ± 0.20^{a}	0.32 ± 0.20^{b}	0.32 ± 0.02^{a}	0.32 ± 0.20
	Scale score	1.98 ± 1.15^{a}	2.68 ± 1.05^{b}	2.36 ± 1.34^{ab}	3.18 ± 0.82^{c}	3.36 ± 0.93^{c}	2.71 ± 1.06

Sample; total number of sample, TL; total length, BH; body height, TW; total weight, CF; condition factor, Body shape; height/length ratio. Values represent mean ± standard deviation; means in the same column with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test. Values not sharing a common or same alphabet letter (a∼d) and they differ significantly at $P < 0.05$.

DISCUSSION

1. Broodstock and F1 generation genetic diversity

In this study, we aimed to improve the Korean Israeli carp (domestic) with increased genetic diversity, a fast growing and fewer scales. We conducted crossbreeding between the Korean Israeli carp and Chinese Songpu mirror carp.

As a result, the average N_A and H_E values of select broodstock of Korean Israeli are 8.3 and 0.743, and F1 is 13.0 and 0.764. The genetic diversity of F1 has been restored compared to the low-diversity of select broodstock of Korean Israeli through breeding between Korean and Chinese. This result was similar in other studies. To improve the genetic diversity of barfin flounder of the endangered species, it was shown that the diversity was improved through crossbreeding (Ortega-Villaizan *et al.*, 2011). But the gene diversity value showed that the F1 offspring samples were slightly lower than the total select broodtock. Some unique genes of the broodstock were considered to be alleles disappeared by bottlenecks over the generation. In this study, natural seed production by group, F1 showed a slightly lower genetic diversity and loss of some unique alleles than selected broodstock. This trend can also be seen in rock bream and golden mandarin fish (Kim *et al.*, 2008; Luo *et al.*, 2015). In this study, seeds were produced by inducing natural spawning at a ratio of 1 : 2. As a result, some broodstocks did not participate in production, resulting in decreased diversity. For all broodstock to participate in seed production, 1 : 1 artificial seed production is a good solution. The average expected heterozygosity (H_E) of the F1 group was 0.764, which was slightly lower than 0.849 of the broodstock (F0). However, it is considered sufficient to maintain genetic diversity of F1 (0.600 or more) (Albenzio and Santillo, 2011). Because, the HE and PIC values for the diversity of F1 were more than 0.600 and more than 0.5. If the PIC of the locus is 0.5 or more, it is judged to have a high polymorphic information amount (Botstein, 1980). In order to maintain the genetic diversity of the next generation, systematic broodstock management and mating scheme should be followed. This is thought to be able to maintain diversity closer to broodstock by performing 1 : 1 artificial seed production.

2. Morphological effects of the F1 groups

Total length and weight were the highest in KC (491.77 mm, 2270.00 g), followed by CC (483.60 mm, 2260.00 g), KK (475.72 mm, 2190.00 g), and CK (471.52 mm, 2160.00 g) groups. The KC group grew faster in 17 months. However, total length of the CK group was small, but body height was similar to that of the other groups and the condition factor was the highest. Scale scores of the F1 group were, 2.08, 2.49, 2.28, and 3.26 for the CC, CK, KC, and KK groups, respectively. The average number of scales crossing between Chinese and Korean (F1, CK, and KC) was 2.39 and broodstock group (F0) was 3.15. F1 showed 0.76 lower scales than the broodstock, indicating that F1 improved the scales. Crossbreeding experiments have showed that different combinations of parents show positive heterosis, which manifests as increased growth rates, survival, and feeding conversion (Bakos, 1995). In order to determine the objective breeding efficiency and to utilize the tank efficiently, it was mixed and kept in a single tank for up to 17 months in the same breeding conditions.

Initial growth had been carried out in the previous study, and the morphological analysis of the 17-month selling period was performed in this study (Hwang *et al.*, 2016). In a previous study, at days 60 and 170 of culture, the length and weight of the CC, CK, and KC group were significantly higher than those in the KK group ($P<0.05$). The CF was also high in the CC and CK group (Hwang *et al*., 2016). Our results of length and weight measurement were higher in the Chinese broodstock group than those in the Korean broodstock group. Generally, the growth rates of fish are influenced by many internal and external factors, though the female broodstock appeared to be more intensely affected (Heath *et al*., 1999; Vandeputte *et al*., 2004). As a result, the initial growth of the CC and CK groups seemed to be similar to that of the Chinese parents due to the influence of Chinese females. Contrarily, the KC group grew faster after 17 months. After 17 months, total length in the CK group was small, but body height was similar to that in other groups and CF was the highest (Fig. 3). This should have a significant effect on the improvement of the merchandise due to the increase in the edible portion. The latter half of growth is largely influenced by environmental factors. KC showed high environmental adaptation. As a result of a comparison of the scale scores of the broodstocks F0 and F1, broodstock F0 was found to have an average of 3.15, while F1 breeding groups (CK, KC) had an average scale score of 2.52. F1 had a better scale condition than the broodstock F0. Through this study, growth, condition factor (body shape), and scales were improved (Fig. 4).

It is essential to consider whether improved breeding traits can cause physiological problems in fish. The Songpu mirror Carp has fast growth, an improved body shape, and few scales than the comparatively scaly German carp (Li *et al*., 2009). According to the study of Hwang *et al*. (2016) on the Israeli carp in Korea, the survival rate was up to 170 days in each group (CC, CK, KC, KK), and the survival rate was high in all groups (>90%) (Hwang *et al*., 2016). In addition, Israeli carp have demonstrated resistance to parasites through releasing mucus in their body saliva. This

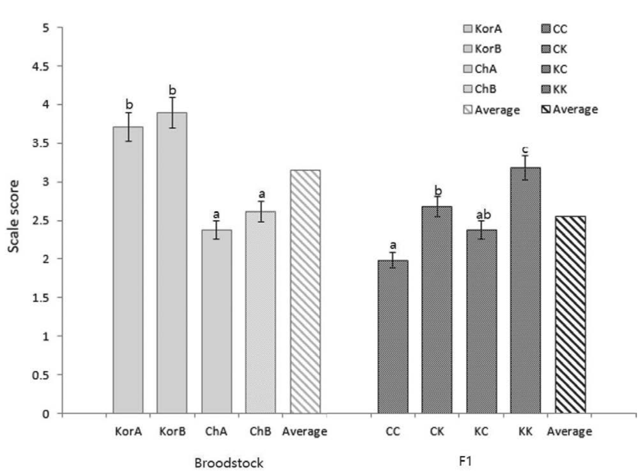

Fig. 4. Comparison of scale the broodstocks (F0) and F1 group.

physiological activity appears to have been an adaption due to reduction of scales. For a more precise study, it is necessary to present objective data through artificial infection trials.

In conclusion, the Israeli carp (F1) developed by the genetic analysis-based cross-breeding method improved genetic diversity, grew quicker, had improved body shape and fewer scales. The improved Israeli carp (F1; CK, KC) in this study grows faster than the common Israeli carp so that it is possible to save both cost and time. The common carp from a general culture farm had been cultivated for two years (24 months) and were sold when the weight was 1.5 kg (Martin, 2007). The common Israeli carp in Korea reached 1.7 kg in 17 months, and improved Israeli carp reached 2.2 kg. The improved carp (F1; CK, KC) had 20% better scales than the parent group (F0), which improved 27% in weight and 25% in scales compared to common Israeli carp. Hence, the improved Israeli carp is more economical than the common Israeli carp. Disease resistance has improved with increased genetic diversity. Further, the body shape was improved and the edible portion also improved. Moreover, improved Israeli carp with low scales, high body shape and high genetic diversity is expected to have high commercial value and adapt well to environmental changes. In future studies, the study on the expression difference of useful genes in each group should be conducted. If improved fish are released into the natural, the impact on the ecosystem has not been investigated. In order to prevent the release of fish, it is considered that the research on the development of the facility reinforcement and future seed production impossible development should be conducted.

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국내 이스라엘 잉어의 선발육종효과 평가

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요 약 : 1973년 이스라엘 잉어(향어)가 한국에 양식을 위해 도입된 이후 현재까지 품종개량에 대한 연구가 전무한 실정이다. 본 연구는 지속적인 근친교배로 인해 낮아진 국내 이스라엘 잉어의 유전적 다양성을 회복하고, 성장이 빠르고 비늘 개선을 위해 유전적 기반 교잡육종 연구를 수행하였다. 본 연구는 한국의 이스라엘 잉어의 품종개량을 위하여 국내 이스라엘 잉어와 중국의 송푸거울 잉어를 이용하여 4개의 교배구를 설정하여 F1을 생산하였다. 친어의 형태 및 유전학적 거리를 고려하여 교배지침을 설정하였다. 본 연구는 유전적 다양성과 친자분석을 위하여 microsatellite 마커와 유전형 데이터를 활용하였다. 그 결과, 국내 친어의 평균 대립유전자와 기대이형접합율은 8.3과 0.743이며, F1은 13.0과 0.764이었다. 국내 이스라엘 잉어와 중국 송푸거울 잉어의 품종 간 교배를 통하여 국내 이스라엘 잉어보다 F1의 유전적 다양성이 회복되었음을 나타내었다. 한국의 일반 이스라엘 잉어는 17개월에 1.7 kg이었고, 개량된 이스라엘 잉어는 2.2 kg에 도달하였다. 또한, KC (한국×중국) 교배그룹의 비늘수치는 2.52, 친어그룹의 비늘수치는 3.15로 나타나 F1은 친어보다 낮은 비늘수치(0.63)를 나타내었다. 품종개량된 이스라엘 잉어(F1; CK, KC)는 친어그룹 (F0)보다 비늘이 20% 개선되었으며, 일반 이스라엘 잉어에 비해 체중(27%)과 비늘(25%)이 향상되었다. 유전적 데이터를 기반으로 개발된 이스라엘 잉어는 상업성이 좋아 국내 이스라엘 양식업에 크게 기여할 것으로 생각된다.

찾아보기 낱말 : 이스라엘 잉어, *Cyprinus carpio*, 유전육종, 마이크로새틀라이트 표지마커, 친자분석, 비늘