

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2022; 37(3): 161-168 • DOI: https://doi.org/10.26650/ASE20221079847

Research Article

Improving the Reproductive Yield of Black Sea Salmon (*Salmo labrax* PALLAS, 1814) with a Selective Breeding Program

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Cite this article as: Cakmak, E., Firidin, S., Aksungur, N., Cavdar, Y., Kurtoglu, I.Z., Aksungur, M., Ozel, O.T., Cankiriligil, E.C., Duzgunes, Z.D., Batir, E. (2022). Improving the reproductive yield of Black Sea Salmon (*Salmo labrax* PALLAS, 1814) with a selective breeding program. *Aquatic Sciences and Engineering*, *37*(3), 161-168. DOI: https://doi.org/10.26650/ASE20221079847

ABSTRACT

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Submitted: 27.02.2022

Revision Requested: 10.05.2022

Last Revision Received: 09.06.2022

Accepted: 10.06.2022

Online Published: 27.06.2022

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This study aimed to improve some culture characteristics of Black Sea salmon (Salmo labrax) culture generations through a classical selective breeding program. Thus, the success of the applied program was examined by comparing the reproduction time and proportional distribution of the wild broodstock (F_{a}) individuals with the F_{a} , F_{a} , F_{a} and F_{a} generation broodstocks adapted to the culture conditions. According to the results, gamete uptake from the new generations occurred between October and February. The highest egg uptake was determined for all generations in December. While the difference between wild (F_0) broodstock and F_1 , F_2 , F_3 , and F_4 generations was statistically significant in favor of new generations (P<0.05), the difference was insignificant between hatchery origin-new generations. Mean egg diameters were low in F_2 and F_3 generations, and F_{n} F_1 and F_4 generations were found to be higher than the others (P<0.05). It was calculated that the fertilization rate was higher in F_3 and F_4 generations, similar in F_1 and F_2 generations, and lower in F_0 generation than the others (P<0.05) at the end of the study. Through the selection program, it was determined that the adaptation of the species to the culture conditions improved, the reaction to human activities declined, and homogeneous distribution in tanks/ponds was relatively achieved from the F, generations. As a result, it has been determined that F, generation broodstock have higher culture performance than other generations. Producers of this species should use F_{A} broodstock for efficient and economical production.

Keywords: Salmo labrax, Black Sea salmon, breeding characteristics, broodstock management, selection program

INTRODUCTION

Black Sea salmon (*Salmo labrax* PALLAS, 1814), also known as Black Sea trout, is an endemic species to the Black Sea from the brown trout family (Tabak et al., 2001). Black Sea salmon, as an anadromous fish, migrate between sea and rivers for reproduction and feeding (Slastenenko, 1956; Svetovidov, 1984; Geldiay & Balık, 1996; Solomon, 2000). The natural distribution area of the Black Sea salmon is the Black Sea and the rivers pouring into it (IUCN, 2016). However, Black Sea salmon is listed as an endangered species according to several local Black Sea countries' databases (GRID, 1999; Lusk et al., 2004; Vassilev & Pehlivanov, 2005; Peev et al., 2011). The decrease in the natural stocks of Black Sea salmon has also directed researchers to aquaculture studies for ex-situ conservation of the species.

The first cultivating study of this species was initiated by the Central Fisheries Research Institute (SUMAE) in 1998. Tabak et al. (2001) created a wild (F_0) broodstock born in rivers in Turkiye, discharged it into the Eastern Black Sea in 1998, and obtained F, progeny in the first reproduction season in the same year. In 2001, a selective breeding program was initiated with studies to determine the species' culture characteristics, with high consumer demand for starting commercial cultivation. When creating the broodstock for each generation, some specific characteristics of the species and culture conditions were considered primary targets. These characteristics were rapid growth, feed utilization efficiency, body form, late maturity (3 years), appearance (silver-spotted coloration for marine ecotype), reproductive efficiency, survival rate, and domestic behavior. After 15 years of research and study, the F₄ generation broodstock was achieved. The private sector supported the study with the F₃ generation broodstock to expand the species' cultivation (Çakmak et al. 2011). Along with these studies, Aksungur et al. (2013) conducted a study called "The use of molecular genetic analyses in stock management of Black Sea salmon." In that study, molecular genetic analyses (microsatellite, sequence analysis of mtDNA genes (cyt-b, dloop and 16S)) were performed on 600 tissue samples taken from 18 private farms producing Black Sea salmon, including SUMAE, and it has been determined that natural genetic variation continues in the broodstock of SUMAE within some farms. With the success of these studies, nowadays Black Sea salmon has great economic importance (Kasapoğlu et al., 2020) due to its rich nutritional value and high consumer demand (Çankırılıgil et al., 2020; 2022). According to the data from the Turkish Statistical Institute, 19 private aquaculture facilities are culturing the species professionally, and 63 private enterprises have trial production permits to raise the species in Turkiye. The total production reached 2311 tons/year (TSI, 2021).

This study discusses the selective breeding program, which plays a significant role in bringing Black Sea salmon into the aquaculture sector and making it a significant part of the seafood industry. The success of the program was examined by comparing the spawning time and proportional distribution of the wild broodstock (F_0) individuals and F_1 , F_2 , F_3 and F_4 generation broodstocks during breeding season, which were caught from some streams in the Eastern Black Sea Region and adapted to the culture conditions. In the literature review that was conducted, no previous study was found regarding the selection program for this species. This research study is the first and most comprehensive broodstock selection study, starting from the wild stock and progressing for 4 generations of Black Sea salmon.

MATERIAL AND METHODS

Sampling studies and adaptation

The wild broodstock individuals were caught from the Black Sea salmon's natural habitats, such as Kapistre, Çağlayan, Fırtına, İyidere, Baltacı, and Solaklı streams. To form the broodstock pool, 3000 fish (\pm 5) at approximately 2+ (34 months) years old were caught and transferred to adaptation units located in Trabzon, Türkiye. Sampling stations and locations of research units are shown in Figure 1. The adaptation study was conducted in the Central Fisheries Research Institute (SUMAE) marine cages research unit, which is composed of 4 m x 4 m square cages with 6 m net depths, in Yomra/Trabzon (salinity 0.17% and water temperature ranging from 5 to 20°C). In this study, 2328 of the 3000 individuals that adapted to culture conditions were used as the first broodstock candidates.

Broodstock selectivity program

In forming F_o broodstock, 650 promising individuals were selected from the 2328 adapted fish, and the first stock was achieved. F, generation juvenile fish were obtained by stripping wild individuals (F_o) in 1998. These juveniles were used in the establishment of the F_1 broodstock. The F_2 generation broodstock was created from the juveniles obtained by stripping 3-year-old F, broodstock in 2001. Individuals obtained from the second breeding season (4 years old) from F_{2} , F_{3} , and F_{4} generation broodstocks were used as the broodstock material for the next generation. Broodstock reaching 7 years of age were removed from the breeding stock (Çakmak et al., 2018). Fish with low sperm quality were not used in the subsequent studies. Sperm quality was determined visually, considering sperm volume and sperm activity via a light microscope. In addition, in each generation, some individuals were discarded from the pool due to the reproductive performance of the previous year (egg yield, gonad size, fertilization ratio, etc.) and observations. New broodstock candidates were added to the stock to maintain the number of 650 individuals in each generation. Finally, some individuals with low body condition and low egg quality who did not develop gonads or developed late gonads in the stripping studies were also excluded from the study. Thus, in this study, the data of 59 females (1360.4±821.06g) and 45 males (1526.8±150.50g) from F₀ broodstock, 167 females (1392.6±780.22g) and 182 males (1526.8±150.50g) from F₁ broodstock, 118 females (1210.8±555.66g) and 136 males females (1252.62±85.45g) from F₂ broodstock, 159 (1663.4±566.87g) and 171 males (1268.44±113.65g) from F₃ broodstock, and 132 females (1252.1±707.01g) and 148 males (1509.6 \pm 122.46) from F₄ broodstock were used. The broodstock selectivity program is shown in Table 1.

Stripping studies and rearing

In stripping studies, firstly, fish were marked using individual markers to determine their reproductive and growth performance. While alphanumeric markers (Visible implant tags, Northwest Marine Technology) were used to mark the F_0 , F_1 , and F_2 generations, electronic markers (Biomark, 12 mm, 134 kHz) were used for the F_3 and F_4 generations in the reproduction season, which spanned October to January. The alphanumeric markers were applied to the transparent tissue above the eyes, whereas electronic markers were applied to the muscle tissue located below the dorsal fin. Markers and the application procedure are shown in Figure 2.

The breeding season of the Black Sea salmon in the natural environment starts in mid-October and continues until the end of December (Tabak et al., 2001). In this study, broodstock control for reproduction started in the first week of October each year following the natural reproduction cycle. Egg maturity controls were made weekly during the breeding season. Individuals that had matured gonads were taken into separate ponds for stripping. Breeding studies were conducted in the SUMAE marine cages research unit (salinity 0.17% and water temperature 5-20°C) and in the stream research unit, which has circular ponds with 6m diameter and 1.2 m depth in the Maçka/Trabzon freshwater unit (water temperature 4-22°C), between 1998 and 2016. Changes in the water temperatures were shown in Figure 3. Broodstock was



Figure 1. Locations of facilities and sampling stations. S: Central Fisheries Research Institute (SUMAE), M: Marine cage unit, F: Freshwater aquaculture unit, S: Sampling stations 1 to 6 (From east to west, Kapistre, Çağlayan, Fırtına, İyidere, Baltacı and Solaklı streams, respectively). The sampling station map was prepared with Ocean Data View software (Schlitzer, 2021).



Figure 2. Markers used in the marking of broodstock and their application areas (a-b: alphanumeric markers, c-d: electronic markers).



research unit and Altındere freshwater unit.

anaesthetized by applying 50 ppm benzocaine (Oswald, 1978) for height-weight measurements and effortless stripping. Stripping was done via the dry stripping method (Billard, 1992). Multiple mating method (3 (F):3 (M)) was applied in fertilization. 25 minutes after fertilization, the eggs were washed with hatching water, and the residues were removed. Fertilized eggs were transferred to the vertical incubators in the freshwater system with the 4-6 egg/cm² stock density at 8-9.5 mg/lt saturated O₂



and 12°C until hatching within 38-40 days. Fry were raised in freshwater units up to smolt length (11.5 cm, 17gr). In the freshwater research unit, water exchange was provided 18-20 times a/ day in the breeding ponds. After stripping, the fish were transferred to marine cage units and kept until June. In June, fish were transferred to the freshwater units due to increasing sea water temperature (\geq 18°C) till the reproduction period. In this stage, *Salmo labrax* reached approximately 30.30±1.63cm in length and 335.50±44.39g in weight in the 8 month span (Çakmak et al. 2007). The stock density was applied as 15 kg/m³ in both marine cages and ponds. The cultivation procedure was carried out according to the study of Çakmak et al. (2010), based on observations gained in domestication studies of the *Salmo labrax*.

Determination of gamete quality and growth parameters

Total length of broodstock was measured with a ruler with a precision of 0.1, while body weight (W) and total egg weights were measured with a precision scale. Total egg weight was determined by weighing the dehydrated eggs for each broodstock. Average egg diameters were determined by scaling 20 eggs for each broodstock in a Von Bayer vessel (Von Bayer, 1910) and calculated by dividing the total number of eggs. Total fecundity was determined using Arıman Karabulut's method (2005). The fertilization rate was calculated by proportioning the remaining eggs (fertilized eggs) to the total number of eggs (Cakmak et al., 2018). Eggs were placed in incubators fed with spring water filled through vertical flow, using separate trays for each broodstock. Eggs that became opaque one day after fertilization were considered unfertilized and were counted and removed. Condition factor was calculated according to Ricker (1975). Commercial trout feed was used for fish consumption. The equations of fecundity, fertilization rate, and condition factor are shown below:

Total fecundity = n/W

n: Total egg count, W: Weight of the individual after stripping (g).

Fertilization rate = $(W_1/W_0) \times 100$ W: Weight (g), L: Length (cm).

Condition factor = $(W \div L^3) \times 100$ W: Weight (g), L: Length (cm).

Statistical analysis

All data were expressed as mean \pm standard deviation (SD). Obtained data were analyzed by performing one-way ANOVA using the SPSS 15 statistical analysis program. Differences between means were compared using Duncan's multiple range test. The homogeneity was determined by the Levene test, while normality was determined by the Anderson-Darling test. Mean values between groups were accepted as statistically significant when the probability value found smaller than 0.05 was accepted as (P<0.05).

RESULTS AND DISCUSSION

The temporal and proportional distribution of $F_{0,}F_{1}, F_{2'}, F_{3'}$ and F_{4} generation broodstocks stripped during the reproduction period is shown in Figure 4. Stripping of wild individuals adapted to the culture conditions (F_{0}) started at the end of October and continued until mid-February. During the reproduction season,



10.17% of the wild broodstock were stripped in October, 22.04% in November, 47.45% in December, 13.56% in January and 6.78% in February (mean values of the years of 1998-2001). The breeding period of F₁, F₂, F₃, and F₄ generation broodstocks was completed in a shorter period than the wild broodstock. F_1 , F_2 , F_3 , and F_4 generation broodstocks were stripped at the rate of 28.74%, 16.94%, 32.08%, and 38.63% in November, 61.07%, 68.64%, 56.60%, and 42.42% in December, 10.17%, 12.71%, 6.91%, and 12.87% in January, 1.79%, and 1.69%, 4.40%, and 6.06% in February, respectively. Brown trout spawn between October and December in the northern hemisphere (Needham, 1945; Horton, 1961; Thomas, 1964; Moyle, 1976). Tabak et al. (2001) stated that the reproductive season of the Black Sea salmon starts in November and continues to mid-December in nature. Salihoğlu et al. (2013) report that the spawning season of the rainbow trout (Oncorhynchus mykiss) is between late December and late February. Similarly, Kurtoğlu et al. (1998) report that stripping of rainbow trout broodstock started in mid-January and continued until the first quarter of March. In this study, stripping of wild individuals (F_a) adapted to the culture conditions started at the end of October and continued until mid-February. The breeding season of F₁, F₂, F₃, and F₄ generation broodstocks started in November and ended in February. F_{4} generation broodstock was mature enough to be stripped at the rate of 38.63% in November, 42.42% in December, 12.87% in January, and 6.06% in February. Unlike the wild population, the stripping season of the F_4 generation broodstock obtained through the selection program starts one month after the stripping season of the brown trout in the northern hemisphere and continues for a long period of four months. The reproduction time of most of the F_4 generation broodstock (81.05%) is between November and December, which does not overlap with the rainbow trout production period. In this case, businesses can have an advantage in using hatcheries. In addition, the preference of compressing the breeding period to a shorter time interval or spreading it over a long period in the natural environment can be possible by implementing good management plans.

Some biological parameters of broodstock individuals are shown in Table 2 and Table 3. Fecundity was found to be lower than oth-

ers in native broodstock (F_n) adapted to culture conditions and similar in F_1 , F_2 , F_3 , and F_4 generation broodstocks (P<0.05). There is no statistical difference between egg yields of F_0 , F_1 , and F_2 generation broodstocks (P>0.05). However, the total egg yield of F₃ and F₄ generation broodstocks were statistically higher than that of the others (P<0.05). The largest egg diameters were observed in F_0 , F_1 , and F_4 generation broodstocks among all groups (P<0.05). Egg yield and size of fish are affected by various factors. The most important of these are broodstock size, age, genotypic structure, and feeding conditions (Bromage et al. 1990, 1992). In salmonids, larger females generally produce larger eggs especially in the culture environment (Sargent et al., 1987; Hatcher et al., 1995). Total egg production and egg diameter values were similar to the values found for wild Black Sea salmon broodstock, but they were different from the other studies mentioned above. In all broodstock groups, total egg production increased in direct proportion to fish size. Heinimaa & Heinimaa (2004) stated that the size of female Atlantic salmon had a positive effect on the total number of eggs, as expected. Furthermore, Şahin et al. (2007) stated that there is a positive correlation between fish size and total fecundity in cultured Black Sea salmon, while a negative correlation exists in wild individuals. In a study by Iwamoto et al. (2017) about selective breeding of the Coho salmon (Oncorhynchus kisutch), egg yield is increasing parallel with weight increase of the individuals, espe-

cially in the 16th and 17th generations. Thus, in this study, the high egg yield of F_{a} and F_{a} generation broodstocks was because the broodstock used was larger than the others. Brown & Kamp (1941) determined the egg yield as 1285 eggs/broodstock, and the egg diameter was 4.64 mm for brown trout in their study. Toledo et al. (1993) studied the reproductive data of 24 brown trout broodstock and found that the egg yield was 1176 eggs/broodstock and the egg diameter was 4.67 mm. Estay et al. (2004) determined the total egg production, relative egg production, and egg diameter of the culture from brown trout as 1904±595 eggs/broodstock, 2591±900 eggs/kg, and 4.77±0.27 mm, respectively. Tabak et al. (2001) report that the total egg yield of wild Black Sea salmon broodstock was 3226±320 eggs/broodstock, the relative egg yield was 1747±70 eggs/kg, and the egg diameter was 5.48±1.10 mm. Serezli et al. (2010) reported that Black Sea salmon has 1404 eggs/ kg total fecundity and 4.51±0.67 egg mm diameter. In another study, total fecundity and egg diameter were determined to be 3524.6±2106.9 and 5.2±0.20 in wild fish and 1931.3±915 and 5.0±0.24 in cultured fish, respectively (Şahin et al., 2007). Our results are similar to other studies.

The fertilization rates of F_1 and F_2 generation broodstocks were statistically the same (P>0.05), and the lowest fertilization rate was determined in the wild broodstock eggs (P<0.05). However, in the F_3 and F_4 generations, fertilization rate was determined to

Table 2.	Some biological parameters of male individuals were used in the study.						
Parameters	Filial Generations						
	F _o (n=45)	F ₁ (n=182)	F ₂ (n=136)	F ₃ (n=171)	F ₄ (n=148)		
L	49.60±1.58	44.8±0.99	46.68±1.04	46.88±1.38	50.4±1.74		
(Min-Max)	(34.8-68.2)	(35.5-56.5)	(35.2-58.6)	(34.7-56.9)	(43.1-59.1)		
W	1526.8±150.50	1526.8±150.50	1252.62±85.45	1268.44±113.65	1509.6±122.46		
(Min-Max)	(562-3603)	(562-3603)	(561-2326)	(479-2207)	(1058-2210)		
CF	1.13±0.01	1.12±0.02	1.19±0.01	1.17±0.02	1.17±0.06		
(Min-Max)	(0.97-1.26)	(0.93-1.27)	(0.99-1.31)	(1.01-1.31)	(1.00-1.64)		
L: mean length (cm) W: mean weight (g) CE: condition factor							

Table 3. Reproduction yields of F_1 , F_2 , F_3 and F_4 generation Black Sea salmon broodstocks.

	Filial Generations						
Parameters	F _o (n=59)	F ₁ (n=167)	F ₂ (n=118)	F ₃ (n=159)	F ₄ (n=132)		
L	49.99±10.24 ^{bc}	50.13±0.78 ^b	47.84±7.44°	53.98±6.17ª	52.48±7.06ª		
W	1360.42±821.06 ^b	1392.60±780.22 ^b	1210.78±555.66 ^b	1663.42±566.87°	1584.25±820.46°		
TEW	248.76±156.05°	294.11±170.29 ^b	236.88±116.01°	348.72±131.75ª	327.40±160.43ª		
EW	0.096±0.01ª	0.096±0.01ª	0.088±0.01°	0.091 ± 0.01^{b}	0.097±0.01ª		
ED	5.46±0.37ª	5.52±0.34°	5.21±0.38 ^b	5.21±0.28 ^b	5.45±0.21°		
EC	2789±1756 ^b	3202±1665 ^b	2916±1472 ^b	3964±1405°	3664±1220ª		
TF	2159±739 ^b	2428±709ª	2512±898ª	2436±593ª	2417±586 ^a		
FR	93.46±5.35°	95.28±6.29 ^b	95.74±4.70 ^b	98.25±1.87ª	98.25±1.81ª		
CF	0.990±0.158ª	1.010±0.087ª	0.988±0.098°	1.021±0.102ª	0.992±0.076°		

L: mean length (cm), W: mean weight (g), TEW: total egg weight (g), EW: the weight of one egg (g), ED: egg diameter (mm), EC: egg count, TF: total fecundity, FR: fertilization rate (%), CF: condition factor. Different letters on the same line indicate the statistical difference in the mean values (P<0.05).

be highest (P<0.05). It is a fact that the fertilization rate of the Black Sea salmon was improved with the selective breeding program throughout the years. In addition, the fertilization rates of F_3 and F_4 generations' eggs were found to be similar to the cultured rainbow trout and brown trout, according to the literature. Tabak et al. (2001) found that the fertilization rate of wild Black Sea salmon eggs is 97.76%. In other research, Estay et al. (2004) reported the highest fertilization rate as 98.5% for cultured brown trout. Salihoğlu et al. (2013) found the average fertilization rate of eggs obtained from rainbow trout broodstock to be 98.7% in a study they conducted in a private enterprise in the Eastern Black Sea region of Turkiye.

Condition factor values were found to be statistically the same in all broodstock groups (P>0.05). Condition factor indicates the general fattening status of the fish, and it can be changed with feeding, gonadal development, and some abiotic factors (Lizama & Ambrosio, 2002). In this study, environmental conditions and feeding regime were kept as constant as possible, and broodstock candidate fish were selected from individuals with the high condition. Therefore, it was expected that there will be no difference between the groups in terms of condition factor values.

CONCLUSION

In the selective breeding programs applied to improve culture characteristics of the species, qualitative characteristics as well as quantitative ones are crucial. In the first generation, some undomestic behaviors, such as escaping from humans, feeding on the bottom rather than the water column, cannibalism, which rises with starvation, and the response to instant changes in the environment (transportation process, salinity, temperature) were improved gradually through the selective breeding study conducted over 15 years. Garner et al. (2010) stated that the breeding method and rearing environment directly affect salmon growth and behavior.

The broodfish displaying an overall reduced sensitivity to environmental conditions are likely to grow faster through feeding more, thereby propagating their traits to the next generation (Solberg et al., 2020). Thus, undomestic behaviors can be reduced with selective breeding programs, as in our study. It was also observed that timid behaviors in the F₀ generation in response to human activities decreased as the generation progressed over time and the adaptation to culture conditions increased. In addition, a relatively homogeneous distribution has been achieved in the tanks/ponds since the F₀ and F₁ generations. General movement of the fish to the feeder's side during feeding and feed intake from the water surface, water column, and bottom were also seen. The second stripping requirement was seen in the first generation broodstock in the stripping season, gradually decreasing as the new generations progressed. Feeding with the appropriate diet and establishing a broodstock from the eggs stripped in the first stripping in selective breeding may be effective for adapting to the culture conditions. Future studies should be carried out to clarify this situation. According to Chavanne et al. (2016), in addition growth and fecundity, feed efficiency, morphology, disease resistance, carcass yield, and product quality are also important traits for the salmonids. With the experience and knowledge gained through this study, future studies on salmon breeding can close the gaps in these areas.

In conclusion, it was determined that the selectivity program carried out with the Black Sea salmon caused a positive effect on the reproductive performance of the fish. Considering the high demand for the Black Sea salmon culture, due to high market value and consumer appreciation compared to other Salmonidae species, the progress gained through this study is vital. Applying a breeding program to improve the culture characteristics of this species, whose culture is spreading rapidly, is essential for business management and profitability. We believe that our findings play a beneficial role in future work, and that the results will interest all researchers while highlighting Black Sea salmon (*Salmo labrax*) as a valuable food source.

Financial Disclosure: Research data for this study was obtained from three different projects, entitled "Black Sea Trout Aquaculture and Using for Restocking (TAGEM/HAYSÜD/2001/07/01/20)," "Introducing Black Sea Trout (*Salmo trutta labrax* Pallas, 1811) to the Private Sector (TAGEM/HAYSÜD/2006/09/03/02)," and "The Determination of Nutritional Requirements in Black sea Trout (*Salmo trutta labrax*) (TAGEM/HAYSÜD/2011/09/01/02)," which were carried out between 2001 and 2015. All projects were funded by the General Directorate of Agricultural Research and Policies (TAGEM), Ministry of Agriculture and Forestry, Republic of Turkiye.

Conflict of Interest: The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Ethics committee approval: Non applicable.

Acknowledgement: We want to thank the directors and staff of our Institute for the support they have provided in infrastructural preparation and usage and technical issues during the study. We also would like to thank Dr. Cemil Altuntaş for his help in writing the article. Once and for all, we would like to express our gratitude to the late Prof. Dr. İbrahim Okumuş, who contributed to creating the selective breeding program.

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