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RESEARCH ARTICLE

PRODUCTION OF HETEROTIC HYBRID IN ROHU (*LABEO ROHITA*) BY CROSSING THE RIVERINE AND HATCHERY STRAINS

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ABSTRACT

As a genetic means of aquaculture development, hybridization can increase production performance quickly in fishes because of heterotic effect. The present study was aimed to produce heterotic hybrid in rohu (*Labeo rohita*) by inter-strain crossing between riverine and hatchery produced strain of rohu, namely cross-1 (Hatchery ♂ x Hatchery ♀), cross-2 (Hatchery ♀ x Riverine ♂), cross-3 (Riverine ♂ x Riverine ♀) and cross-4 (Hatchery ♂ x Riverine ♀) as well as the growth performance of the spawns was compared with the parental strains for a period of 10 weeks in the earthen nursery rearing ponds of Khulna University, Khulna, Bangladesh. The riverine strain showed the lowest fertilization and hatching rates (83% and 61%) compared to hatchery strain and reciprocal hybrids. In the hybrid, a positive heterosis of 25.51% was obtained. Growth performance study revealed that both the reciprocal hybrids and riverine strain performed better than the hatchery strain. Nevertheless, mean total length among the crosses did not show any significant difference. Since the growth rate of strain crossed hybrid was reasonably high, this technique can be profitably applied for the production of fast growing seed for successful aquaculture venture.

Key words: Heterosis, Inbreeding, Fertilization and Hatching Rate, Growth Performance.

INTRODUCTION

Fish culturists show special interest on natural fish seed due to their rapid growth rate, high disease resistance and survival rate. The hatchery produced seed shows lower growth and survivability probably due to inbreeding problem in hatcheries. The genetic variation of hatchery population is generally lower than that of wild populations because hatchery populations are necessarily produced from a limited numbers of parents, negative selection and smaller brood size at first maturity (Shah, 2004). Further, the seed producers do not pay attention to the genetic quality of the seed for enhancing production performance (Hussain and Mazid, 2005). Shah (2000) indicates research needs in fish breeding and genetics in Bangladesh and points out the problems on genetic management of the brood stock in the hatcheries in Bangladesh. Hence, it has become essential to carry out research on genetic improvement of the species to conserve the genetic diversity in natural fish populations which is eroding very fast due to anthropogenic stresses as well as aquaculture productivity requires for further enhancement to meet the growing animal protein demand, particularly of fish protein. It is anticipated that the application of genetics would contribute substantially in improving aquaculture productivity. Hybridization can bring quick change through exploitation of heterosis. It can give good outcome if the species (strains) hybridized is inbred.

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These inbred will then provide heterotic effect. Hybridization is practiced to achieve two major goals either to obtained heterosis or hybrid vigor among the progeny above the average of the parental performances or value and/or non-heterotic effects which is the performance of the progeny as the result of simple combination of parental genotypes. Heterosis achieved through inter-specific and/or intra-specific strain crossing enables offspring to surpass its parents for one or more traits. Heterosis is achieved in F_1 generation through individual loci interaction (Yan and Ozgunen, 1993). Hybridization may also be used to transfer other desirable characteristics from one group or species to another, to combine valuable traits from two species, and to produce sterile individuals.

Hybrids may also be used to exploit degraded aquatic environments (Bartley *et al.*, 2004). On the whole, hybridization within species (crossing between different strains) often produces good heterosis (David and Pandian, 2006). In the present experiment, attempts were made to produce heterotic hybrid in rohu through strain crossing between riverine and hatchery originated broods. Growth performance was tested for the reciprocal intra-specific crosses. Growth performance of riverine and hatchery crosses showed significantly higher performance than the rest of the crosses. The result of the experiment clearly indicated that the broods should be collected from divergent sources to maintain the genetic quality of the hatchery produced seed in Bangladesh for successful aquaculture venture.

MATERIALS AND METHODS

Brood Collection And Transportation: the sexually matured riverine brood fishes were collected from "Boluhor Fish Hatchery" under Jhenaidah district, Bangladesh. The origin of the brood fishes was the two major rivers of Bangladesh namely, the Padma and the Jamuna. The broods were transported in large metallic tanks with supplied aeration.

Brood Stock Maintenance: the collected brood fishes were stocked and reared in the brood rearing ponds of "Fish Seed Multiplication Farm (Gallamari, Khulna)", under the Department of Fisheries, Government of the People's Republic of Bangladesh. Before stocking in the ponds, the riverine broods were kept in a conditioning tank for 24 hours with shower and continuous water flow. The broods were tagged by fin clipping at the base of the dorsal fin and were treated with 5 ppm $KMnO_4$. Supplementary feed was given at a rate of 5% of total biomass during the rearing period at ponds.

Induced Breeding And Intra-Specific Hybridization: The brood fishes were collected from brood stock ponds of the hatchery and kept in conditioning tanks for 12 hours. Then the broods were shifted for induced breeding into a concrete brood tank provided with continuous shower and water recirculation system. The mature male and female of rohu were taken and inseminations were performed for intra strain crossing with reciprocal cross-inseminations. Induced breeding was performed by using carp pituitary hormone injection intramuscularly. The doses were 2mg/ kg and 6mg/ kg body weight for female administered at 6 hour duration while male was given only one injection at 2mg/ kg body weight at the time of second dose to the female. Eggs and milt were collected by hand stripping and were mixed well in clear plastic bowl for proper fertilization with the help of feathers. Mature ovulated eggs of female were fertilized with the sperm of normal male. Eggs and sperm were collected separately before mixing. Physiological saline solution (5% dextrose and 0.9% NaCl) was used during fertilization to increase the viability of eggs and sperm and also to reduce the sticky condition of eggs. Four different crosses were produced with the single strain crossing and intra-specific hybridization of rohu i.e. cross-1 = Hatchery ♂ x Hatchery ♀, cross-2 = Hatchery ♀ x Riverine ♂, cross-3 = Riverine ♂ x Riverine ♀, cross-4 = Hatchery ♂ x Riverine ♀.

Incubation of Eggs: The fertilized eggs were incubated in round hatching jars of 50 liter water holding capacity connected to water circulating system. A continuous flow of fresh water was maintained in the incubation jars for gentle movement of the developing eggs to enhance proper hatching of eggs. Thereafter, the fertilized eggs were separated from the unfertilized eggs by the presence of transparent shell with gray spot within the egg shell, while the unfertilized eggs were opaque. The fertilized eggs were counted and rate was determined by using the following equation:

Fertilization rate (%) = (No. of fertilized eggs/Total no. of eggs) X 100

To count hatching rate exactly, 100-200 fertilized eggs were isolated and kept in small hatching jars of 6 liter water holding capacity with continuous flow of fresh water. Then the actual hatching rate was determined by counting the number of hatchlings in the jars.

Eggs were hatched within 20 to 24 hours. The larvae were reared in the jars for three days. Supplementary feed, egg yolk was given in the jars twice daily after 24 hours of hatching.

Pond Preparation: The earthen ponds of each 1.25 decimal were used to observe the growth performance of the fishes. Ponds were sun dried for several days and lime was applied at a dose of 2 kg decimal^{-1} of CaCO3. Further, cow dung, urea and TSP were applied at a rate of 30 kg decimal^{-1}, 500 g decimal^{-1} and 250 g decimal^{-1} respectively. The ponds were fenced with small mesh sized net to avoid the entrance of predatory and undesirable species in the ponds. After liming and fertilization the ponds were filled with water (rain water) to a depth of about 2 meter. Within 3-4 days after fertilization, the water of the experimental ponds attained green color, indicating that adequate plankton had been produced.

Stocking of the Larvae in the Ponds: The 3 day old larvae were stocked in 8 earthen ponds. The stocking density of the larvae was 2,500 decimal^{-1}. Four different treatments were used in the present experiment, each were replicated for twice to compare growth performance. Before releasing in water, acclimatization of fry in the new environment was done to reduce mortality.

Feeding: During the experimental period, the fish larvae were fed with supplementary feed (Progoti nursery feed, containing 30% crude protein) twice in a day at the rate of 8% of the estimated biomass for the first 4 weeks and then at the rate of 6% of the estimated biomass for the rest of the rearing period.

Growth Performance: To assess the growth performance and feed adjustment, fishes were sampled randomly at fortnightly interval. Growth performances (both length and weight) were checked for a period of 10 weeks. In total, 8 different ponds were used to compare the growth performance of 4 different crosses. Weight was measured by using an electric balance (AND GF-300, Japan) and length was measured by using a centimeter scale. During the first sampling, length was not measured due to the smaller size of the larvae.

Heterosis Calculation: Heterotic effect was calculated by using the following equation

H= (Mean reciprocal F1 hybrids – Mean parents) X 100 / Mean parents

Water Quality Monitoring: dissolved oxygen (DO), temperature (at 30 cm depth), pH, hardness and alkalinity were measured and analyzed weekly. Water temperature and dissolved oxygen (DO) were measured by using oxygen meter (Hanna Instrument H1 9143). The pH was measured by using pH meter (EUTECH Instrument cyber-scan pH-11). Standard titrimetric methods (Greenberg *et al.* 1992) were used to analyze hardness and alkalinity. Furthermore, transparency was recorded with Sacchi disk.

RESULTS

Average fertilization rate of four different fish groups are shown in the Figure 1. Cross-1 showed the significantly higher fertilization rate (92%) than Cross-2 and Cross-3. The average hatching rates of the four different crosses are presented in the Figure 2.

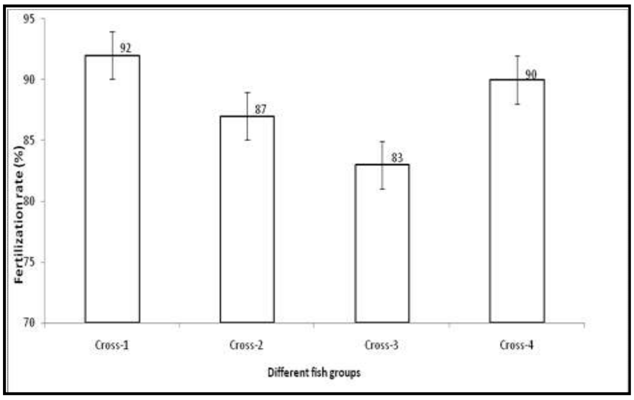

Figure 1. Average fertilization rates of four different fish groups

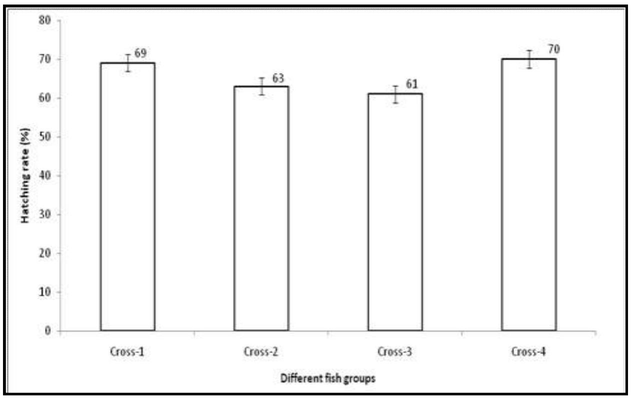

Figure 2. Average hatching rate among four different crosses

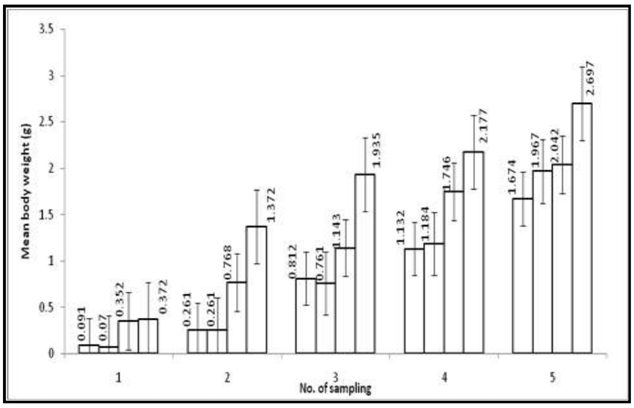

Figure 3. comparative growth trial between different fish groups in terms of mean body weight

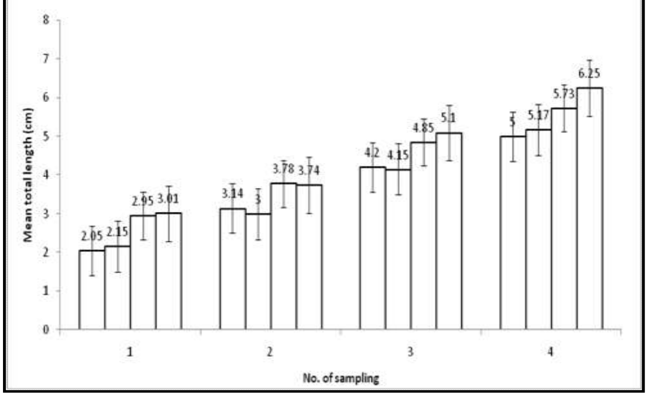

Figure 4.Ccomparative growth trial between different fish groups in terms of mean total body length

During the experiment, Cross-4 showed the highest hatching rate where as Cross-3 showed the lowest rate (figure 2). There was a significant difference on the average hatching rates between controlled crosses (cross-1 and 3) and between treated crosses (Cross-2 and 3). The comparison of growth performance showed significant differences ($P= 0.039< 0.05$) in the mean body weight among the different fish groups twice in a month; providing 25.51% positive heterosis (figure 3). Nevertheless, mean total length did not show significant differences (figure 4). Furthermore, the experiment was conducted in eight earthen ponds and water quality was assessed weekly (Table-1).

Table 1. Ranges of water quality parameters of the rearing ponds

Parameters	Value	
	Range	Mean ± SD
pH	6.5-7.5	7.2 ± 0.3
Temperature (°C)	27-32	30 ± 1.6
Transparency (cm)	25-36	29.4 ± 5.3
DO (mgl^{-1})	5.8-6.9	6.2 ± 0.4
Hardness (mgl^{-1})	180-250	210 ± 20
Alkalinity (mgl^{-1})	220-255	235 ± 15

DISCUSSION

Fertilization Rate and Hatching Rate

The intra-specific hybrids (controlled Cross-3) showed significantly lower fertilization rate (83%) compared to control cross 1 (92%), probably due to the change of its habitat that created extra environmental stress and further added stress during transportation. The average fertilization rate for the hybrid of hatchery and riverine strain was obtained at 75.75%, for riverine strain 75.49% and for hatchery strain 65.49% (Islam and Shah, 2007). The intra-specific hybridization of rainbow trout provided better fertilization performance than that of the parental members (Moav and Hulata, 1975) but for channel catfish the fertilization of intra-specific crosses was lower compared to the parents observed by Wohlfarth *et al.* (1975) and Hulata *et al.* (1985). The Cross-1 and 4 showed significantly higher hatching rates over the Cross-2 and 3. Islam and Shah (2007) obtained 71.3% hatching rate from riverine strain, 41.8% hatching rate from hatchery strain and 64.5% hatching rate from hybrid strains of rohu. The intra-specific crosses showed higher hatching percentage in common carp (Bakos and Gorda, 1999; Wang, 2009), Chinook salmon (Barman *et al.* 2003), tilapia (Tave *et al.* 2007), catla and mrigal (Biswas *et al*. 2008). Lower hatching rate was also observed in intra-specific hybrids of guppy (Julie and Yoshihisa, 1988), cutthroat trout (Allendorf and Leary, 1984), Arctic charr (Nilsson, 2005), African catfish (Wachirachaikarn, 2009). In the present experiment, the average fertilization and hatching rate for all the crosses were comparatively higher, indicating better management practice, good physiological and genetic conditions of the brood fishes and may also be due to the well mixing of eggs and sperm with physiological saline solution (5% dextrose and 0.9% NaCl) by using a feather that probably increased the viability of gametes.

Growth Performance

Cross-4 (Hatchery ♂ x Riverine ♀) showed the best growth performance over all the crosses.

Therefore, the maternal effect of riverine strain on growth performance was reflected better than the female of hatchery strain. The best growth performance of Cross-4 could most probably ascribed to the increased heterozygosity in the offspring produced from crossing of riverine female and the hatchery produced male. The growth performance of riverine strain (Cross-3) was also found better than the hatchery strain (Cross-1); indicating the slowest growth rate of hatchery strains probably due to accumulation of inbreeding and small effective number of broods. On the other hand, total body length increment was recorded insignificant among all the crosses. Islam and Shah (2007) noticed better growth performance in the reciprocal intra-specific hybrids of hatchery and riverine strains of rohu. They also noticed that "riverine female x hatchery male" had comparatively better growth increment. The results of the present experiment supported the findings of the results of Islam and Shah (2007). Biswas *et al.* (2008) obtained better growth performance of riverine strains of Indian major carps over the hatchery strains. Dunham and Smitherman (1983) reported 55% increase in growth rate of channel catfish intra-specific hybrid and Dunham (1996) reported 22% increase in growth rate of rainbow trout crossbred.

The rate of inbreeding (F) in India is particularly high for rohu, catla and mrigal (Eknath and Doyle, 1990). Shah (2004) reported that the hatchery produced seed of the Indian major carps have significantly lower growth rate than the seed originated from the natural sources of river in Bangladesh. The lower growth performance of hatchery stocks indicated improper or poor management practice of stocks in hatcheries and ignorance of genetic aspects on the part of hatchery workers (Allendorf and Phelps, 1980; Ryman and Stahl, 1980). In Bangladesh, almost all the hatchery facilities do not have adequate number of ponds to raise their brood fishes (Biswas *et al.* 2008). The hatchery operators usually maintain a small effective number of broods (N_e), do not maintain their pedigree records and thus produce adverse effects on the gene pool of hatchery stock (Allendorf and Uttar, 1979; Tave and Smitherman, 1980; Ryman and Stahl, 1980; Cross and King, 1983 and Tave, 1999).

The additive genetic variation (V_A) is created by the cumulative action of alleles across the loci and Tave (1993) suggested that in an inbred population with very little amount of V_A or no V_A present at all, making it difficult to bring changes in production performance through selection, the only option that is left to increase production is hybridization. Through hybridization, dominant genetic variation (V_D) is exploited. V_D is attributed to the dominant action of alleles between individual loci. In the present study, inbred hatchery strain of rohu was mated with the riverine strain of the species in order to increase heterozygosity through exploitation of V_D. There are available information on the use and applicability of strain crossing for increased vigor of the offspring produced (Moav *et al.* 1974; Moav and Wohlfarth, 1974; Kirpichnikov, 1981; Wohlfarth, 1993; Bakos and Gorda, 1995 and Biswas *et al*, 2008). However, strain crossing of riverine and hatchery produced rohu revealed 25.51% positive heterosis in the intra-specific hybrids. Islam and Shah (2007) obtained 55.76% positive heterosis in the hybrids by crossing hatchery and the riverine river strains of rohu. The intra-specific (strain crossing) technique provided 29.84% positive heterosis in common carp (Wang, 2009), 38% positive heterosis in Chinook salmon (Barman *et al.* 2003), 17% positive heterosis in tilapia (Tave *et al.* 2007), 78% positive heterosis in guppy (Julie and Yoshihisa, 1988), 13% positive heterosis in cutthroat trout (Allendorf and Leary, 1984) and 28% positive heterosis in African catfish (Wachirachaikarn, 2009). The intra-specific hybridization technique may also provide negative result or slower growth performance than the parental strain as it was found in Arctic charr (Nilsson, 2005), where 15% negative heterosis was obtained.

Water Quality Parameters

Different water quality parameters i.e. temperature, dissolved oxygen (DO), pH, hardness, alkalinity and transparency were measured weekly and found very suitable throughout the experimental period (Passy, 2007; Krebs, 2001; Romesh and Anbu, 1996).

Conclusion

The present experiment was successful in producing heterotic hybrid in rohu (*Labeo rohita*) through strain crossing between riverine and hatchery strains achieving 25.51% positive heterosis. The results obtained from this investigation revealed that the intra-specific crossed or strain crossed rohu seed performed significantly better than both the pure hatchery strain and pure riverine strain seed in terms of growth performance. In order to produce more fish seed with the growing feed demand, special attention must be given to maintain a large stock of broods, broods should be collected from divergent sources, the pedigree record must be kept and better management practice must be performed in hatcheries to produce good quality seed for increased production. This can reduce the chance of inbreeding and bottle neck effect in hatcheries. Furthermore, the intra-specific hybridization or strain crossing technique can effectively be introduced to the inland aquaculture of Bangladesh to boost aquaculture production.

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