Patterns of colour inheritance from crossbreeding between Red hybrid tilapia (*Oreochromis* sp.) and GIFT tilapia (*Oreochromis niloticus*)

O.F. Nwachi^{1,3}, Y.B. Esa^{1,2*}, A. Christianus¹, A.A. Rahim¹ and M.S. Kamarudin¹

¹Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, 43400, Malaysia

²International Institute of Aquaculture and Aquatic Sciences (I-AQUAS), Universiti Putra Malaysia, Negeri Sembilan, 71050, Malaysia

³Department of Fisheries, Delta State University Abraka, Asaba Campus, P.M.B. 1, Nigeria

*Corresponding Author Email : yuzine@upm.edu.my

Paper received: 22.11.2020

Revised received: 23.01.2020

Accepted: 14.08.2020

Abstract

Aim: To examine the pattern of colour inheritances from crossbreeding between Red hybrid tilapia (*Oreochromis* sp.) and GIFT tilapia (*Oreochromis niloticus*).

Methodology: A total number of 60 broodstock of 30 each of GIFT and Red hybrid tilapia were selected to produce the first filial generation (F_1) and its reciprocal nF_1 . Pairing of female to male were done in the ratio of 3:1. The same breeding scheme were used to produce the second filial generation (F_2) and its reciprocal $nBcF_1$.

Results: Crossbreeding between Red hybrid tilapia male and GIFT female (F_1) produced phenotypic colours ranging from red (64.61%), wild type (11.54%), red with blotches (4.96%) and mixed red-wild type (18.87%). The reciprocal cross between GIFT male and Red hybrid tilapia female (nF_1) produced phenotypic colours ranging from red (19.65%), wild type (64.62%), red with dark blotches (4.03%) and mixed red-wild type (11.46%). The second filial (F_2) generation produced phenotypes comprising of 61.17% wild type, 14.37% red with dark blotches, 20.04% mix red-wild type and 4.40% red type. The backcross (BcF_1) produced 10.57% faded wild type 22.76%, red with dark blotches, 28.41%, red with dark posterior and 60.46% red phenotype while the reciprocal ($nBcF_1$) produced 52.89% faded wild type, 15.11% red with dark blotches, 6.83% mix red-wild type and 25.15% red phenotype.

Interpretation: Manifestation of intermediate and mixed colour phenotypes in F_2 hybrids and deviation from a simple dominant inheritance might indicate an incomplete dominant inheritance, colour determination in tilapia in this study might be located or linked with the male Y-chromosome due to the fact that more reddish coloured fish were produced when Red hybrid tilapia was selected as the parent.

Key words: Colour phenotype, Crossbreeding, Red hybrid tilapia, GIFT, Mendelian inheritance

Breeding scheme of crossbreeding between Red tilapia and GIFT tilapia

How to cite : Nwachi, O.F., Y.B. Esa, A. Christianus, A.A. Rahim and M.S. Kamarudin: Patterns of colour inheritance from crossbreeding between Red hybrid tilapia (*Oreochromis* sp.) and GIFT tilapia (*Oreochromis niloticus*). *J. Environ. Biol.*, **41**, 1289-1294 (2020).

Introduction

Tilapia is regarded as a fish with great potential for aquaculture because of its ability to reproduce in captivity, accept supplementary feed and can be easily crossbred or hybridized (Benhaïm *et al.*, 2017). The production of GIFT (Genetically Improved Farm Tilapia) strain is an example of highly successful selective breeding programme utilizing genetic variability and heritability information of population level broodstocks of a single species (*Oreochromis niloticus*) (Hamzah *et al.*, 2014). On the other hand, crossbreeding or hybridization among different tilapia species, either naturally or through artificial propagation has resulted in the production of various strains of Red hybrid tilapia (Watanabe *et al.*, 2002; Rajaei *et al.*, 2010).

Although the initial genetic records on the production of Red hybrid tilapia is not well documented, their derivation is generally attributed to crossbreeding between a mutant reddish orange *O. mossambicus* with other species including normal *O. aureus*, *O. niloticus*, and *O. hornorum* (Watanabe *et al.*, 2002). The aims are generally to obtain other desirable traits such as faster growth rate or higher tolerance towards cold temperature or salinity (Rajaei *et al.*, 2010). Nowadays, Red hybrid tilapia strain is one of the most popular tilapia cultures in most countries in Southeast Asia and make up to 85% of total tilapia production in Malaysia (Ng and Hanim, 2007).

GIFT strain has an estimated cumulative increase of 85% in growth rate as compared to the base population and exhibits superior traits; fast growth rate, quality fillets and higher survival rate (Beardmore *et al.*, 2001). Nowadays, GIFT strain is distributed worldwide, and is gaining popularity in less developed and developing countries (Trevas, 1982). Nevertheless, the only drawback of GIFT strain is its wild type coloration (greyish black) which is considered less favorable as compared to the red hybrid strain by customers in certain parts of the world (Southeast Asia and South America). Accordingly, the Red hybrid tilapia is preferred over the wild type or black colored tilapia due to its attractive and clean-like red coloration, its near resemblances to reef or marine fishes and its ability to fetch a premium price of USD4.20 per kg in fresh fish market.

Studies on the pattern of inheritance of colour and pigmentation in tilapia has been documented by (Thodesen *et al.*, 2013; Pongthana *et al.*, 2010 and Rajaei *et al.*, 2010), however, the molecular mechanism responsible for the determination of colour and pigmentation is still poorly understood. Accordingly, most of the red genotype of Red hybrid tilapia strains are predicted to be controlled by a single dominant trait (either homozygous dominant RR or heterozygous Rr), while the recessive wild type is homozygous recessive (rr) (Wohlfarth *et al.*, 1990; Huang *et al.*, 1988). This includes the Thai strain, Philippine red and Sterling strain. Meanwhile, the Taiwanese and Fijian strains both exhibit pink body colouration for the homozygous

dominant RR allele (Rajaei *et al.*, 2010). In addition, the red colour is often associated with spots or blotches (mainly black but also red) which makes the fish less attractive and affects their market prices. In *O. niloticus*, these blotches are hypothesized to be mainly associated with the heterozygous red fish which carry the Rr genotype, however, the genetics underlying this trait is still not fully understood (Rajaei *et al.*, 2010; McAndrew *et al.*, 1988).

Therefore, this laboratory scale partial diallel crossbreeding experiment was conducted between the GIFT strain (*O. niloticus*) and a Red hybrid tilapia strain (*Oreochromis* sp.) that had been cultured and maintained at Universiti Putra Malaysia hatchery for more than 15 years with the objective to determine the genetic pattern of colour inheritance of reciprocal crosses over two generations and to examine the influence of sire gender selection on the phenotype of the offspring.

Materials and Methods

Broodstock selection : A total number of 60 tilapia fish broodstocks consisting of 30 of each GIFT and Red hybrid tilapia, making up to 48 females and 12 males each, were selected as sires to produce the first filial generation (F_1) and its reciprocal nF_1 . The GIFT strain brood stock was obtained from World Fish Centre (Penang) and maintained in the hatchery for about three generations. Meanwhile, the Red hybrid tilapia strain was originally obtained from two private hatcheries and was also maintained and cultured in the hatchery for more than 10 years. The hatchery record stated that the Red hybrid tilapia strain was a product of hybridization between *O. niloticus* and *O. mossambicus*.

The production of first filial generation (F_1) was done by reciprocal mating between GIFT and Red hybrid tilapia sires. Selected F_1 dams harbouring only the red body colouration were subsequently selected for the next generation breeding experiments. Accordingly, the F_1 was self-crossed (female F_1 with male F_1) to produce the second filial generation (F_2), the backcross (BcF_1) was produced by crossing Red hybrid tilapia female with F_1 male while the $nBcF_1$ was produced by crossing GIFT female with F_1 male. All fish collections and breeding experiment were conducted and maintained at the UPM Aquaculture Research Station in Puchong, Selangor, Malaysia.

Experimental design : A fish tank of one tonne capacity was used for spawning. The female to male ratio for the breeding experiment was 3:1. A total of 3 selected females of each tilapia strain were stock-up in triplicates per experimental unit, commercial feed was given for a period of 14 days to allow acclimatisation and the greening of water. The male mouths were clipped to reduce the adverse effects of male aggression by the copulating pair. Each experimental units was modelled in triplicate. The males were introduced into the units housed with females after they might have assumed territories and their

stomach filled with matured eggs that spilled out on gentle massage of the stomach.

The water was allowed to go green to reduce any distraction in the tank from external environment sources. Three pieces of 4 inches (10.16 cm) diameter and 1 m length of PVC pipe were placed into the tank to serve as a hiding place for the copulating fish. The fries were assumed to hatch out at the time they were observed at the sides of the tank because of the green water that provide privacy during courtship and fertilization. After hatching, the parent stocks were immediately removed from the hatching tank, the male fish were placed in a solitary tank for two weeks before re-stocking. While swim up fries were reared in the experimental brooding tanks with a partial change of water.

Results and Discussion

Hybridization has been widely applied as a technique to improve the traits or phenotypes of tilapia in order to make them more desirable in the market (Bentsen *et al.*, 2017; Nwachi and Esa 2016; Neira *et al.*, 2015). Thus, the current crossbreeding study between GIFT and Red hybrid tilapia and its reciprocals, the backcrossing of respective parents to their F_1 and production of F_2 by self-crossing of F_1 were conducted with the aim to produce a putative hybrid GIFT tilapia harbouring the red colouration genotype from the Red hybrid tilapia.

Although, the genetic assessment of red body color in tilapia is not fully understood, the red colouration in *O. niloticus* and most red hybrid tilapia are believed to be controlled by a dominant-inheritance (Lago *et al.*, 2017; Reich *et al.*, 1990; Ferreira 1989). Accordingly, the red body colour in *O. niloticus* is controlled by a single dominant allele where both homozygous RR or heterozygous Rr produce red body colour while the normal wild type (dark) are controlled by a recessive allele (rr) (Reich *et al.*, 1990). Therefore, in theory, hybridization between a Red hybrid tilapia carrying a dominant red allele (RR) with a GIFT (rr) is expected to produce all F_1 red colour phenotypes while hybridization between a heterozygous Red hybrid tilapia (Rr) with GIFT (rr) is expected to produce 1 red :1 wild type colour ratio phenotypes. A phenotypic ratio of 3 red:1 wild type is expected from the offspring of F_2 generation, while the back cross (Bc F_1) of F_1 generation with the red parent is expected to produce a 3 red: 1 wild-type ratio while the backcross of F_1 generation with the GIFT parent is supposed to produce 1 red: 1 wild-type phenotypic ratio.

The results of the crossbreeding study over two generations (F_1 , F_2 and backcrosses) produced progenies consisting of red and wild type body colour phenotypes. Furthermore, the red colour can be further subcategorized into two body colour patterns; red and red with dark blotches, while the wild type colour can be subcategorized into two body colour

Table 1 : Percentage and average of colour phenotypes of first filial (F_1) generation and its reciprocal crosses between Red hybrid tilapia and GIFT tilapia

Mating		Genotype of parents				Colour				Ratio
♀	♂	Rep.	N	♀	♂	Red		Wild-type		Red: Wild-type
						Red	Red with dark blotches	Mixed red-wild type	Wild-type	
GIFT	Red	1	292	rr	Rr	136	14	86	56	1:1
		2	232	rr	Rr	107	12	67	48	1:1
		3	323	rr	Rr	150	16	87	70	1:1
					Total		435		414	1:1
Red	GIFT	1	281	Rr	rr	84	44	30	123	1:1
		2	306	Rr	rr	90	53	35	128	1:1
		3	329	Rr	rr	99	52	42	136	1:1
					Total		422		494	1:1
Red	Red	1	321	Rr	Rr	200	45	0	76	3:1
		2	320	Rr	Rr	198	44	0	78	3:1
		3	341	Rr	Rr	206	52	0	83	3:1
					Total		745		237	3:1
GIFT	GIFT	1	311	rr	rr	0	0	0	311	0:1
		2	324	rr	rr	0	0	0	324	0:1
		3	343	rr	rr	0	0	0	343	0:1
					Total		0		978	0:1

Rep = Number of replicates, N= Offspring number

patterns; the typical wild type (grey black) and a diluted black with slightly red blotches (identified as mixed red-wild type) as shown in Table 1 and Fig. 1 (a-d). In fact, the 1 red: 1 wild type ratio found in the F_1 generation indicated that the red hybrid parent selected for the study may carry the heterozygous Rr genotype. On the other hand, the red with dark blotches pattern can be categorized as exhibiting the dominant Rr phenotype as well, since blotching is presumed to be genetically associated with the " r " gene in the red tilapia strain (Rajaei *et al.*, 2010). Meanwhile, the mixed red-wild type colour pattern presumably carried the same recessive rr genotype with the wild type colour. The control crosses of the Red hybrid female X Red hybrid male produced a 3 red: 1 wild type ratio, while the control GIFT female X GIFT male produced a 0 red: 1 wild type ratio, in accordance with the Mendelian inheritance pattern.

The crossing of first filial generation (F_1) which produced the F_2 backcross BcF_1 and reciprocal $nBcF_1$ also produced four colour phenotypes similar to the F_1 hybrid generation. However, the percentages of the observed phenotypes differed depending

on the paired crosses. The self cross between first filial generation ($F_1 \times F_1$) produced the second filial generation (F_2) offspring with 43.91% red type, 28.67% red with blotches, 5.04% mix red-wild type and 22.38% wild type colour (Table 2).

On the other hand, the backcross mating (BcF_1) between Red hybrid tilapia and F_1 hybrid produced backcross generation comprising of 44.13 % red type, 30.87 % red with dark blotches, 5.87 % mix red-wild type and 19.23 % red colour phenotype. Meanwhile, the reciprocal crossing between GIFT with the F_1 hybrid produced 34.42% red type, 15.52 % red with dark blotches, 12.25 % mix red-wild type and 37.79 % wild type phenotype (Table 2).

Nevertheless, the intermediate (mixed red-wild type) colour pattern observed in the F_1 hybrids, F_2 hybrids and backcrosses raised question on whether the pattern of inheritance truly follow the simple Mendelian dominant-recessive pattern commonly found in Red hybrid tilapia as suggested by previous studies (Wohlfarth *et al.*, 1990; Reich *et al.*, 1990; Rajaei *et al.*, 2010; Lago *et al.*, 2017).

(a) Red

(b) Red with dark blotches

(c) Mix red-wild type

(d) Wild type

Fig. 1 (a-d) : The four colour patterns from reciprocal crossbreeding between Red hybrid tilapia and GIFT tilapia.

Table 2 : Percentage and average of colour phenotypes of second filial (F_2), backcross (BcF_1) and its reciprocal ($nBcF_1$)

Mating		Genotype of parents				Colour				Ratio
♀	♂	Rep.	N	♀	♂	Red		Wild-type		Red: Wild-type
						Red	Red with dark blotches	Mixed red-wild type	Wild-type	
F ₁	F ₁	1	298	Rr	Rr	135	90	10	63	3:1
	F ₂	2	355	Rr	Rr	144	95	26	90	3:1
		3	300	Rr	Rr	139	88	12	60	3:1
					Total	691	261	3:1		
Red	F ₁	1	280	Rr	Rr	120	90	15	55	3:1
	BcF ₁	2	345	Rr	Rr	159	100	20	66	3:1
		3	311	Rr	Rr	134	99	19	59	3:1
					Total	702	234	3:1		
GIFT	F ₁	1	305	rr	Rr	107	45	30	123	1:1
	nBcF ₁	2	326	rr	Rr	113	50	43	120	1:1
		3	335	rr	Rr	117	57	47	127	1:1
					Total		489		490	1:1

Rep= Number of replicates, N= Offspring number

The intermediate (mixed red-wild type) colour phenotype found in this study over two generations suggested that a simple dominant-recessive inheritance system may not be applied. The deviation indicated that the colour inheritance may be controlled by other non-Mendelian system since the dominant red allele (RR or Rr) produced an intermediate phenotype (mixed red-wild type) in a heterozygous form (Rr). According to the hatchery record, the Red hybrid tilapia stock reared in UPM hatchery was produced through hybridization between *O. mossambicus* with *O. niloticus*, however, the exact strain (whether a Thai strain, Philippine red strain or Taiwanese strain) could not be verified based on the record alone.

The fact that F_2 and backcrosses (BcF_1 and $nBcF_1$) all produced mixed red-wild type colour phenotype suggested an incomplete dominant pattern of inheritance for the current GIFT-red hybrid crosses. Huang *et al.* (1998) inferred the red body color of *O. mossambicus* x *O. niloticus* and even in Taiwanese Red hybrid tilapia was inherited as a single gene with incomplete dominance. Nevertheless, Lovshin *et al.* (1990) opined that colour determination in tilapia is complicated because of the diverse genetic make-up of Red hybrid tilapia hybrids and colour variations of red that have been described among off-spring from a common female. Additionally, some colour phenotypes are sub-viable and some phenotypes change colour over time making genetic determination of colour complex.

The proportion of reddish coloured phenotypes in F_1 hybrid, however, differed between reciprocal crosses depending on the male parental species used for the experiment. A high

percentage (average 46.28%) of red coloured type hybrid were observed when male Red hybrid tilapia was selected as broodstock, but low percentage (average 29.80%) of red type coloured hybrid were found when male GIFT tilapia (wild type colour) was used as broodstock. Likewise, a high percentage of hybrid fish exhibiting wild type colour phenotype was observed when GIFT Tilapia was assigned as a male parent (42.25%) when compared with the Red hybrid tilapia male (20.49%) in the study.

Therefore, the results of this study suggested that the gene responsible for colour determination in tilapia might be located or linked with the male Y-chromosome, where a red hybrid tilapia male presumably would produce higher percentage of reddish coloured offsprings as compared to wild type male parent. A related study by Parnell and Steelman (2013) using quantitative trait loci analysis of restriction site-associated DNA -tag single nucleotide polymorphisms (SNPs) indicated the chromosomal linkage between the orange blotch colour and ZW chromosome in tilapia, which further supported the assumption.

In conclusion, the crossbreeding experiment between Red hybrid tilapia and GIFT tilapia was carried out successfully under laboratory conditions, and the colour inheritances of F_1 , F_2 and backcrosses hybrids were examined. The outcomes of this study showed that crossbreeding between the two tilapia species produced an intermediate phenotype (mixed red-wild-type) which indicate incomplete dominant inheritance pattern. Overall, the results of this study would be useful and important for conducting and managing proper breeding scheme and propagation of F_1 hybrid and/or later generation hybrids either in a laboratory, intensive or even extensive hatchery scales.

Acknowledgments

The authors would like to thank all the staff of the Department of Aquaculture, Faculty of Agriculture, UPM and lab mates from Fish Genetics and Breeding laboratory, who have assisted in this project. The authors would also like to take this opportunity to thank Universiti Putra Malaysia for the opportunity to conduct the research. The study was part of the PhD thesis of O.F. Nwachi.

References

- Beardmore, J.A., G.C. Mair and R.I. Lewis: Monosex male production in finfish as exemplified by tilapia: Applications, problems, and prospects *Aquaculture*, **197**, 283–301 (2001).
- Benhaim, D., D.D. Akian, M. Ramos, S. Ferrari, K. Yao and M.L. Bégout: Self-feeding behaviour and personality traits in tilapia: A comparative study between *Oreochromis niloticus* and *Sarotherodon melanotheron*. *Appl. Anim. Behav. Sci.*, **187**, 85–92 (2017).
- Bentsen, H.B., B. Gjerde, A.E. Eknath, M.S.P. de Vera, R.R. Velasco, J.C. Danting, E.E. Dionisio, F.M. Longalong, R.A. Reyes, T.A. Abella, M.M. Tayamen and R.W. Ponzoni: Genetic improvement of farmed tilapias: Response to five generations of selection for increased body weight at harvest in *Oreochromis niloticus* and the further impact of the project. *Aquaculture*, **468**, 206–217 (2017).
- Ferreira, J.: On the inheritance of the red colour in the Mozambique Tilapia *Oreochromis mossambicus*. *MADOQUA*, **1**, 5–8 (1989).
- Hamzah, A., R.W. Ponzoni, N.H. Nguyen, H.L. Khaw, H.Y. Yee and S. Mohd Nor: Performance of the genetically improved farmed tilapia (GIFT) strain over ten generations of selection in Malaysia. *Pert. J. Trop. Agri. Sci.*, **37**, 411–429 (2014).
- Huang, C.M., S.L. Chang, H.J. Cheng and I.C. Liao: Single gene inheritance of red body coloration in Taiwanese Red hybrid tilapia. *Aquaculture*, **74**, 227–232 (1988).
- Lago, A. de A., T.T. Rezende, M.A.D. Dias, R.T.F. Freitas and A.W.S. Hilsdorf: The development of genetically improved Red hybrid tilapia lines through the backcross breeding of two *Oreochromis niloticus* strains. *Aquaculture*, **472**, 17–22 (2017).
- Lovshin, L.L., D. Tave and A.O. Lieutaud: Growth and yield of mixed-sex, young-of-the-year *Oreochromis niloticus* raised at two densities in earthen ponds in Alabama, U.S.A. *Aquaculture*, **89**, 21–26 (1990).
- McAndrew, B.J., F.R. Roubal, R.J. Roberts, A.M. Bullock and I.M. McEwen: The genetics and histology of red, blond and associated colour variants in *Oreochromis niloticus*. *Genetica*, **76**, 127–137 (1988).
- Neira, R., X. García, J.P. Lhorente, M. Filp, J.M. Yáñez and A.M. Cascante: Evaluation of the growth and carcass quality of diallel crosses of four strains of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **451**, 213–222 (2015).
- Ng, W.K. and R. Hanim: Performance of genetically improved Nile tilapia compared with red hybrid tilapia fed diets containing two protein levels. *Aquaculture Res.*, **38**, 965–972 (2007).
- Nwachi, O.F. and Y.B. Esa: Comparative Growth and survival of diploid and triploid Mozambique Tilapia (*Oreochromis mossambicus*) reared in indoor tanks. *J. Environ. Biol.*, **37**, 839–843 (2016).
- Parnell, N. and J.T. Streelman: Genetic interactions controlling sex and color establish the potential for sexual conflict in Lake Malawi cichlids. *Heredity*, **110**, 239–246 (2013).
- Pongthana, N., N.H. Nguyen and R.W. Ponzoni: Comparative performance of four Red hybrid tilapia strains and their crosses in fresh- and saline water environments. *Aquaculture*, **308**, 109–114 (2010).
- Rajaei, A.H., F.A. Huntingford, K.J. Ranson, B.J. McAndrew and D.J. Penman: The effect of male colouration on reproductive success in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, **308**, 119–123 (2010).
- Reich, L., J. Don and R.R. Avtalion: Inheritance of the red color in tilapias. *Genetica*, **1**, 195–200 (1990).
- Thodesen, J., D. Ma, M. Rye, Y. Wang, S. Li, H.B. Bentsen, M.H. Yazdi and T. Gjedrem: Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, survival and external color traits of Red hybrid tilapia (*Oreochromis spp.*) after four generations of multi-trait selection. *Aquaculture*, **417**, 354–366 (2013).
- Thodesen, J., M. Rye, Y.X. Wang, S.J. Li, H.B. Bentsen, M.H. Yazdi and T. Gjedrem: Genetic improvement of tilapias in China: Genetic parameters and selection responses in growth, survival and external color traits of Red hybrid tilapia (*Oreochromis spp.*) after four generations of multi-trait selection. *Aquaculture*, **416**, 354–366 (2013).
- Trevas, E.: The biology and culture of tilapias ICLARM Conference proceedings. In: (Eds.: R.V.C. Pullen and R.H. Lowe-McConnell). *Tilapias; taxonomy and speciation*. No 7 ICLARM Manila Philippines, pp. 3–13 (1982).
- Watanabe, W.O., T.M. Losordo, K. Fitzsimmons and F. Hanley: Tilapia Production Systems in the Americas: Technological Advances, Trends, and Challenges. *Rev. Fish. Sci.*, **10**, 465–498 (2002).
- Wohlfarth, G.W., S. Rothbard, G. Hulata and D. Szwedman: Inheritance of red body coloration in Taiwanese tilapias and in *Oreochromis mossambicus*. *Aquaculture*, **84**, 219–234 (1990).