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# Inbred zebrafish lines: A genetic repository for zebrafish researchers

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**Abstract**

**Genetically uniform strains/inbred strains of animals are used in experiments that demand minimal genetic background variability and highly reproducible results. Modern genomic research is heavily dependent on inbred strains and they are widely used for identification of quantitative loci that affect evolutionarily and biomedically important traits, dissection of gene-gene and gene environmental interaction patterns, toxicity studies, immunology, reverse genetics, cancer research, etc. In mice, over 450 inbred strains have been described and in medaka over 13 inbred strains have been established. Owing to its numerous attributes zebrafish, *Danio rerio*, is one of the widely studied vertebrate model organisms, but comparable zebrafish inbred strains are not available to the scientific community. This is because of early onset of inbreeding depression causing high mortality of embryos and larvae and biased sex ratio that have been reported from earlier attempts to produce inbred lines. At CZeBraG-Centre for Zebrafish Breeding and Genetics Research facility of ICAR-CIFE, the inbred lines of zebrafish are being developed. The strategy followed is intense inbreeding coupled with strong positive selection for selected traits. The base population consisted of three wild stocks of zebrafish collected from rivers of North East India. Using nested mating design 84 families were produced in $F_1$ generation and 46 inbred families were produced in $F_2$ generation. Genetic parameters for growth traits like body weight and standard length were estimated. The overall heritability for body weight and standard length was estimated using animal model and found to be 0.43 ± 0.05 and 0.36 ± 0.04 respectively. Fecundity was also recorded but showed no significant variation among stocks. Inbreeding depression was studied by comparing the population means for body weight and standard length of $F_1$ and $F_2$ generation. The analysis suggested there was no inbreeding depression. Genetic monitoring of zebrafish is conducted using microsatellite markers. New PCR primers were designed for 20 selected microsatellite loci with high stringency. High Resolution Melting Curve analysis was used for genotyping individual zebrafish. In addition to developing inbred lines the centre is also maintaining the wild zebrafish stocks and has commenced supply of pedigreed zebrafish for researchers. In future zebrafish of known inbred levels (12.5 to 50% and above) can be supplied. Currently, the CZeBraG can house about 10000 adults, with separate facility for holding individual fish.**

**Keywords**: Zebrafish, inbreeding, inbred strain

## Introduction

An inbred strain is a population of animals that result from a process of at least 20 sequential generations of brother-sister matings. The resultant animals are essentially clones of each other at the genetic level (Silver and Abbot 1996; Broughton 2002). More than twenty Nobel prizes have been awarded for work that probably would have been impossible without inbred strains (Festing 2014). Just as the purity of the chemical assures the pharmacist of the proper filling of the doctor's prescription, so the purity of the mouse stock/inbred can assure a research scientist of a true and sure experiment. C. C. Little in 1936 mentioned, in experimental medicine, the use of inbred genetic material is as necessary as the use of aseptic and anti-septic precautions in surgery (Festing 2007). Inbred strains are genetically homogeneous populations, and are powerful experimental tools that are ideally suited for studying immunology, cancer, and genetics of complex traits (Shinya and Sakai 2011). Genetically homogeneous strains also provide essential tools for the identification of quantitative loci that affect evolutionarily and biomedically important traits (Frankel 1995; Kimura et al. 2007; Klingenberg et al. 2004, 2001; Tomida et al. 2009; Xiao et al. 2010). The key properties of inbred strains are: Isogenicity, Homozygosity, Long term stability, Identifiability and Individuality (Festing et al. 2002). Modern genomic research is heavily dependent on these strains. Inbred

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strains of mice, rats and guinea-pigs have been available for nearly a century. In mice, over 450 inbred strains and in Medaka over 13 inbred strains have been established (Steven et al. 2007; Shinya and Sakai 2011).

The zebrafish, *Danio rerio* (Teleostei infraclass and Cyprinidae family) has emerged as an excellent vertebrate model organism for studies related to genetics, immunology, developmental and cancer biology, etc., due to excellent traits that facilitate genetic analyses like external fertilisation and development, optical clarity during embryogenesis, high fecundity, short generation times and ease of maintenance (Zhoa et al. 2015). In India, zebrafish is found in Brahmaputra, Gandak, Rapti, Karnal, Kali, Ramganga, Ganges, Yamuna and Indus river drainages, Nadhave and Kalauma rivers in Kumaon Himalayas, Balangi district, Orissa Western Ghats, Kabini River, Thunga River and Wynaad district of Kerala (Hora 1937; Chauhan 1953; Menon 1962; Tilak 1968; Parichy 2015). Even though zebrafish is available in plenty in wild and is a favorite model organism for geneticist, very limited breeding studies have been conducted in India.

The Japanese group, Shinya and Sakai (2011) and Shinya (2016) developed two inbred zebrafish wild-type strains, Tubingen and India (IM) through full sib-pair mating. An inbred Tubingen strain failed to thrive and was lost after 13 generations. An inbred India strain (IM) has been maintained successfully. The IM strain has endured 16 generations of inbreeding and has maintained a healthy condition. The zebrafish is one of the widely studied vertebrate model organisms but comparable zebrafish inbred strains are not available globally to the scientific community (Shinya and Sakai, 2011). Our study reports the first inbreeding program of zebrafish in India. At ICAR-CIFE, Mumbai, India, a National Facility for Zebrafish Genetic Resource named CZeBraG-Centre for Zebrafish Breeding and Genetic Research, has been established. The goal of CZeBraG is to conduct genetics research and serve as a genetic repository for inbred and wild lines of zebrafish.

## Materials and methods

### *Collection of wild zebrafish stocks*

The zebrafish was collected from natural waters of West Bengal-(River Mahananda-Darjeeling, River Tista-Jalpaiguri and River Raidhak), Assam (River Manas, Manas National Park) and Tripura (Muhri River). The stocks collected were assembled and are maintained at CZeBraG (Centre for Zebrafish Breeding and Genetic Research) facility present at ICAR-CIFE (Central Institute of Fisheries Education), Mumbai, India.

### *Breeding*

Pure breeding of zebrafish was carried out to generate full sib and half sib families for two generations. To obtain the known level of inbreeding a planned nested mating is followed. Mating pairs were chosen based on phenotypes for body weight and length. In the evening hours the mating pair was housed in a glass aquarium with marbled bottom for preventing feeding on eggs by parents and it enhances resemblance with natural habitat. Fish usually spawn next day in the early morning hours after few minutes of exposure to light. The families were reared separately at a stocking density of 2 fishes/litre of water in equal sized glass aquariums.

### *Recording of traits*

Growth traits *viz*., standard length and body weight were recorded at 120 days age post fertilisation. All the measurements were taken after anesthetizing the fish by adding a few drops of clove oil to 10 L of tank water. Before weighing, the fishes were wiped off with a piece of soft tissue paper. The standard length was measured with the help of laminated semi log graph sheet (having a minimum reading of 0.05 cm). Body weight was recorded with a standardized digital weighing balance in terms of milligram as the unit. Adult zebrafish exhibits distinct sexual dimorphic morphology. The females are larger sex with rounded belly and males are slender and smaller. Fecundity of each selected female parent was recorded after each spawning. Soon after spawning the mating pair was removed from the tank and the eggs were siphoned off, washed and placed in a petridish and counted by naked eyes.

### *Genetic parameters*

The heritability was estimated by following univariate linear mixed animal model and genetic correlation between the traits was estimated by employing SAS PROC MIXED procedure (Saxton 2004).

### *Estimation of inbreeding depression*

Inbreeding depression was studied by comparing the means for body weight and standard length of $F_1$ and $F_2$ generation (for both full sibs and half sibs). Test for differences between F1SL ($F_1$ Standard Length), FsSL

($F_2$ Full sib Standard Length), F1BW ($F_1$ Body weight), FsBW ($F_2$ Full sib Body weight), HsBW ($F_2$ Half sib body weight), HsSL ($F_2$ Half sib Standard length), CbSL (Cross Bred Standard length) and CbBW (Cross Bred Body weight) was estimated to know the inbreeding depression.

### *Designing of PCR primers*

New PCR primers (Table 1) with high stringency for microsatellite loci were designed for 20 selected microsatellite loci using Gene Runner v 3.0. The length of the primers was 22-26 bases, Tm was 45°C to 65°C

**Table 1.** Information on microsatellite loci and primers

| Name | LG | Type | Location | Repeat | Primers sequence | Expected size(bp) | Annealing temp. |
|---|---|---|---|---|---|---|---|
| Z13685 | 10 | Di | Exon | (AC)20 | AAACTGCAATTTAACCAGTGGGAT<br>GGCACAATGAAGATCAAGGTAAACATC | 131 bP | $65^0$C |
| Z4329 | 20 | Tri | Intron | (TCA)7 | CACAGAGAGAGGCGGGAAATA<br>CAGGGAGAATGAAATGCTTGCAATCCG | 105 bp | $65^0$C |
| Z13253 | 7 | Tri | Intron | (TTG)8 | TGAGTGAATGTGTGCAGAGGAT<br>CGGCATTTGAGATGTTGATGTTGACCTG | 150 bp | $65^0$C |
| Z7490 | 4 | Di | Intron | (TG)38 | CGGGCAACGAAATGACGAGGA<br>GACGACGGGCACAACTGTCTTGG | 180 bp | $65^0$C |
| Z7632 | 2 | Comp | Intron | (CA)20(GC)8 | TTCAAGCCAGTCTGATGGAACATTT<br>G TGGACAGAAGGTCGTGACTGCATG | 194 bp | $65^0$C |
| Z13685 | 10 | Tetra | Exon | (ATGA)3 | TGCGGCCATCCACTGCATAAAG<br>CATTGACTGTGCGGTGGACTGAAACTC | 131 bp | $65^0$C |
| Z21115 | 8 | Di | Exon | (CA)40 | AAATTTGGCATCCTTGCTTTGGTCA<br>CCCTCATCTGTGGCTCACGTTCTGC | 132 bp | $65^0$C |
| Z720 | 23 | Comp | NC | (CA)40(TCT)3 | CAGCAACCTATCACTGGGAGACATCC<br>CACAGGGAATGAGCTGAGAACAGGAG | 324bp | $65^0$C |
| Z9511 | 16 | Comp | NC | (ATG)3(GA)8<br>(AGG)3 | CAGGCTGAGGCAGAGGAATTTGTTG<br>TTACTTGAAGCTAGCCTGCACGTCC | 267bp | $65^0$C |
| Z1412 | 13 | Di | NC | (TG)40 | ATGCAGATCAGGCTGTGAATGTTC<br>AGTTTGTGTGAATGTTCAATTCTCAGGG | 309bp | $61^0$C |
| Z4325 | 12 | Di | NC | (TG)24 | GTCCCAATCCCTTCTGCCCTCAC<br>TAGCGTTTCCTCCAGAGACTCCAGCAC | 212bp | $63^0$C |
| Z7807 | 9 | tetra | NC | (AACA)4 | TTCAGATCGTCATCAGTTGTGGG<br>TGTGGATGAACTGGCTTGTCATG | 149 bp | $65^0$C |
| Z1525 | 4 | Di | NC | (AC)19 | ACGGTGCTGAATCACGGCTGATAAA<br>CGCGTCAGACTGAAGATCCTCTTGCTG | 214 bp | $65^0$C |
| Z4349 | 2 | Tri | NC | (ATG)15 | TGGGTAATGAAGCGTTTCTGTGGT<br>GCGGAGATCAGCTTTGAGGTCAGAATC | 159 bp | $65^0$C |
| Z20966 | 3 | Di | Both | (CA)10 | ACAACACTCAGCACCAGAACAGACCG<br>GCCTTCAGCTTTAGTGGTTCAGTCCC | 168 bp | $65^0$C |
| Z5022 | 16 | Tetra | Intron | (AATG)4 | GGTTACAGCTGAAAGGGCGTCGT<br>CAATGTTGGAAACTGGAAGCCATTGG | 186 bp | $65^0$C |
| Z9871 | 5 | Di | Intron | (TG)25279 | TTTGACAATGCACAGCCCACATC<br>ATTGTTTATTCTCCTTGCGGAGTGAG | 235 bp | $65^0$C |
| Z10324 | 22 | Di | Exon | (TG)15 | AGAGATGCCTGCCTGCCTTACAGATC<br>AGAGGAGCCACGATTGATGGAGG | 142 bp | $65^0$C |
| Z10215 | 11 | Tri | NC | (ATC)8 | TGATCAGGTGTCGTCCTCAGCGTG<br>TGTTCTGCTGTTTAGACCATGTGACG | 186bp | $65^0$C |
| Z10551 | 1 | Tri | NC | (ATT)16 | CAGAAACGCCAACTGAGTCAGT<br>CGGAACAGGTAAACACCAGAGAGCG | 115 bp | $65^0$C |

NC = Non Coding

and GC content was 40 to 60%. Primers were searched for the absence of dimerization, hairpin formation and secondary priming sites to avoid mispairing. Tm difference between primer pairs was <5°C. Polynucleotide stretch was avoided. The primers were standardised using touchdown PCR. The zebrafish genomic DNA was extracted by the standard Phenol-chloroform method and PCR was performed in 25 µl reaction volume containing 50 ngtemplate DNA, 10 pmol of each specific primer, 200 µM of each dNTPs, 0.75 units of Taq DNA polymerase and 1× Taq buffer containing 1.5 mM MgCl2 (Sambrook et al. 2001). The amplification reaction was carried out in 0.2 ml PCR tubes in a heated lid thermocycler. The PCR conditions included initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing for 20 s using touch down conditions set at 65°C to 60°C, extension at 72°C for 30 s and final extension at 72°C for 8 min. The amplified products were visualised on 1.5% agarose gel.

### *Genetic monitoring of zebrafish*

Genotyping of zebrafish was done by High Resolution Melting Curve analysis that was carried out in Roche Light cycler 96 using MeltDoctor™ HRM Master Mix (Thermoscientific, USA) following manufacture's protocol. The results were analysed using the instrument's software. Each reaction was performed in 20 µl reaction volume (Table 2). The amplifications were carried out in Roche Light cycler 96 (Table 3). The results were analysed using the instrument's software. The normalised melting curve and heat map

**Table 2.** Reaction mixture for High Resolution Melting

| Components | Volume for one 20-µL reaction | Final concentration |
|---|---|---|
| MeltDoctor™ HRM Master Mix | 10 µL | 1X |
| Primer 1 (5 µM) | 1.2 µL | 0.3 µM |
| Primer 1 (5 µM) | 1.2 µL | 0.3 µM |
| Genomic DNA (20 ng/µL) | 1.0 µL | 1 ng/µL |
| Deionized water | 6.6 µL | — |
| Total volume | 20 µL | |

was used to genotype the samples and the putative alleles were scored for each locus. The shape of the melting curve depends on the GC content, length and sequence of the amplicon.The various curve shapes could be grouped into single inflection point graphs for homozygous samples and graphs with two inflection points for heterozygous (Wu et al. 2008, Ganopoulos et al. 2011).

**Table 3.** PCR stages for High Resolution Melting

| Stage | Step | Temperature | Time |
|---|---|---|---|
| Holding | Enzyme activation | 95°C | 10 min |
| Cycling | Denature | 95°C | 15 sec |
| (40 cycles) | Anneal/extend | 60°C | 1 min |
| Melt curve/ | Denature | 95°C | 10 sec |
| dissociation | Anneal | 60°C | 1 min |
| | High resolution melting | 95°C | 15 sec |
| | Anneal | 60°C | 15 sec |

## Results and discussion

### The genetic repository: CZeBraG- Centre for Zebrafish Breeding and Genetic Research - A National Facility for Zebrafish Researchers

The CZeBraG facility is developed at ICAR-Central Institute of Fisheries Education, Mumbai. The facility was inaugurated by honourable DG ICAR-Dr Trilochan Mohapatra. At the facility there are eight hundred glass aquariums of various sizes for housing zebrafish. Individual zebrafish are placed in labelled aquariums to maintain their pedigree records. Each family is reared separately in larger aquariums at a stocking density of 2 fish/ litre. Filtered and UV treated water is supplied to the rearing system. An indigenously developed artemia hatching set up is installed for zebrafish feeding. Zebrafish are reared at 28°C at a 14 h light: 10 h dark cycle. Water quality parameters *viz*., temperature, pH, ammonia, nitrite and nitrate are monitored regularly. Water temperature is maintained at 26°C±2 and pH at 7-8. Aeration is provided in all the tanks. Different types of feed is given three times a day based on the age of fish (1-12 days old larvae are exclusively fed with live paramecium, 12- 24 days old larvae are fed with both paramecium and live Artemianaupli, 24-40 day old juveniles are fed with Artemia alone, >40 days old fishes were fed with Artemia – one time and Tetra Bit®/flakes-2 times a day). One week prior to breeding, brood fishes are fed four times a day with artemia (twice) and tetra bits flakes (twice). Left over feed materials is siphoned manually and water exchange is done regularly. Moribund/diseased and dead fishes are quarantined and removed from facility. Currently, pedigreed zebrafish from the facility are supplied to various researchers on demand

### *Breeding*

Pure breeding of zebrafish within stocks was carried out to generate full sib and half sib families for two generations. Using nested mating design 84 families across stocks were produced in $F_1$ generation and 66 inbred families in $F_2$ generation were produced using $F_1$ parent. Genealogical F was estimated using Proc Inbreed procedure of SAS. A cross bred population comprising of 12 families was also generated by inter se mating among the stocks. There were a total of 1516 observations for body weight and standard length. The average body weight and standard length of the total population is 195±1.87 mg and 2.17±0.01 cm, respectively. Among stocks, West Bengal population had highest mean for body weight and standard length 215±3.65 mg and 2.32±0.02 cm, respectively, followed by Assam and Tripura. Gender-wise the females had higher body weight and standard length measuring 243.23±2.58 mg and 2.29±0.01 cm, respectively (Table 4). The average body weight and standard length of $F_1$ generation was 197.11±2.22 mg and standard length was 2.21±0.01 cm, respectively. The average body weight and standard length of $F_2$ generation was 191.46±3.40 mg and 2.08±0.02 cm, respectively. The average body weight and standard length of cross bred progeny was 237.11±12.72 mg and 2.46±0.06 cm, respectively.

In the present study it was observed that female zebrafish were longer and heavier as compared to male zebrafish. The reproductive status demands that females need to be larger in size to help them produce larger and more eggs. Earlier studies in zebrafish also reported that females tend to be larger than males both in domesticated and wild populations (Eaton and Farley 1974; Spence and Smith 2007). The body weight showed more dispersion with a CV of nearly 27%. The high dispersion in body weight is a common phenomenon in fishes. This large variation may be due to differences in stocking density experienced by each family at early stage of life, the tendency of few individuals to grow much faster than others even under optimum culture conditions and also an inherent difference in the growth rate of female and males. Compared to body weight the variation in standard length was less with CV being around 15%. The average body weight and standard length of zebrafish in the present study was much less than the earlier reports (Eaton and Farley 1974; Spence and Smith 2007). The difference in these averages may be due to differences in feeding regime and effects of domestication. It is also reported that the natural populations when bred in captivity tend to grow slower in the first few generations as G x E effect may be playing a role.

Both genetic and non-genetic factors influence the growth traits significantly. In the present study the gender, stock, family, and the interaction between them were considered as the major source for variation in standard length and body weight. The analysis of variance showed that the gender of the fishes, stock, interaction between gender and stock and gender and family had significant effect on both the traits.

### *Genetic parameters*

There was between and within family variation for standard length and body weight (Figs. 1a and 1b) in zebrafish. The heritability was estimated by following mixed animal model. The heritability of standard length and body weight was 0.43±0.05 and 0.36±0.04, respectively. The genetic correlation between standard length and body weight was r = 0.88±0.05. The moderately high and precise values of heritability suggest the presence of genetic variation in growth

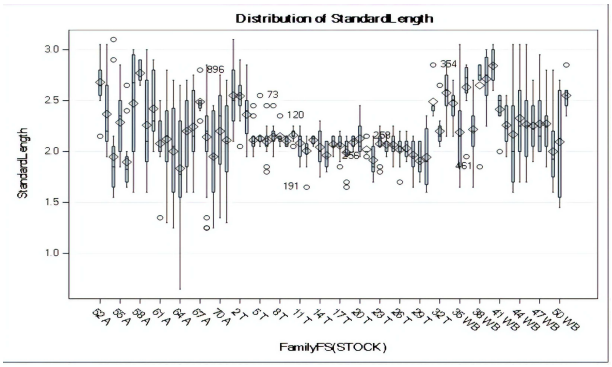


**Fig. 1a. Between and within family variation for standard length**

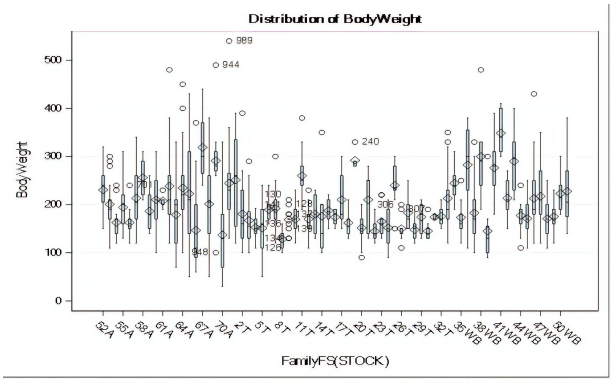


**Fig. 1b. Between and within family variation for body weight**

**Table 4.** Stock-wise and gender-wise descriptive statistics for body weight and standard length in zebrafish

| Variable | Body Weight (mg) | | | | | Standard Length (cm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Min | Max | Mean | SE | CV | Min | Max | Mean | SE | CV |
| Assam | 663 | 30.0 | 550.0 | 202.67 | 3.04 | 38.63 | 0.65 | 3.15 | 2.17 | 0.02 | 19.54 |
| Tripura | 514 | 20.0 | 390.0 | 172.22 | 2.75 | 36.15 | 0.95 | 3.10 | 2.06 | 0.01 | 15.34 |
| West Bengal | 339 | 90.0 | 480.0 | 215.46 | 3.65 | 31.19 | 1.45 | 3.05 | 2.32 | 0.02 | 15.55 |
| Female | 670 | 70.0 | 550.0 | 243.23 | 2.58 | 27.49 | 0.65 | 3.15 | 2.29 | 0.01 | 16.40 |
| Male | 846 | 20.0 | 350.0 | 157.17 | 1.78 | 32.90 | 0.95 | 3.05 | 2.07 | 0.01 | 17.95 |
| Overall | 1516 | 20.0 | 550.0 | 195.21 | 1.87 | 37.27 | 0.65 | 3.15 | 2.17 | 0.01 | 17.90 |

traits of zebrafish and the scope to alter them by appropriate genetic selection methods. The higher heritability in the present case is expected as the base population is a mixture of the three geographically distinct stocks which may have brought a large genetic variation in the population. The high heritability may also be due to a large number of fishes were full-sibs and the model may not have succeeded in separating the dominant effect from additive genetic effect. In the present case each family was reared separately and this may have also had a confounding effect with the additive genetic effect and may have inflated the heritability estimates. The estimated heritability values obtained in our study are similar to other studies conducted in food fishes like trouts (McKay 1986; Perry 2005; Gjerde et al. 1994, Quinton et al. 2005, Bongers et al. 1997) and guppy (Nakajima and Fujio 1993). The genetic correlation estimated for body weight and standard length was strong and positive (0.88±0.003). The high genetic correlations (r,) between these two traits indicate the existence of pleiotropy among additive genes i.e., the same set of genes determining both these traits (Falconer, 1998). The genetic correlation value obtained in our study is similar to values obtained for other fishes like those reported earlier in salmonids (Refstie 1980), Arctic char (Nilsson 1992) and Rohu (Gjerde and Reddy 1996).

### *Inbreeding depression*

The analysis of traits suggested that there was no inbreeding depression in the $F_2$ generation. An unintentional inbreeding usually leads to inbreeding depression. In the present study, the $F_2$ generations were produced by mating full sib and half sibs. This resulted in the birth of progenies with 25 and 12.5% inbreeding. This high level of inbreeding is expected to bring down the growth performance. However, in the present case the parents of $F_2$ generation were selected for their growth traits. The selection differential for body weight and standard length of parents used to produce $F_2$ full sibs was 40 mg and 0.25 cm and for parents used to produce $F_2$ half sibs was 74 mg and 0.21 cm, respectively. The differences in $F_1$, $F_2$ and

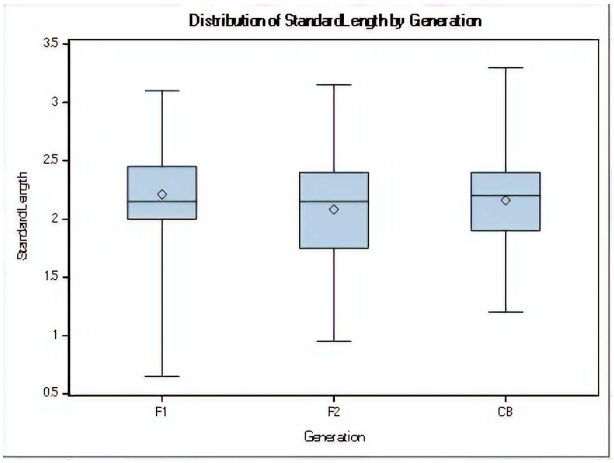


**Fig. 2a. Box Plot of standard length for $F_1$, $F_2$ generation and Cross Bred population**

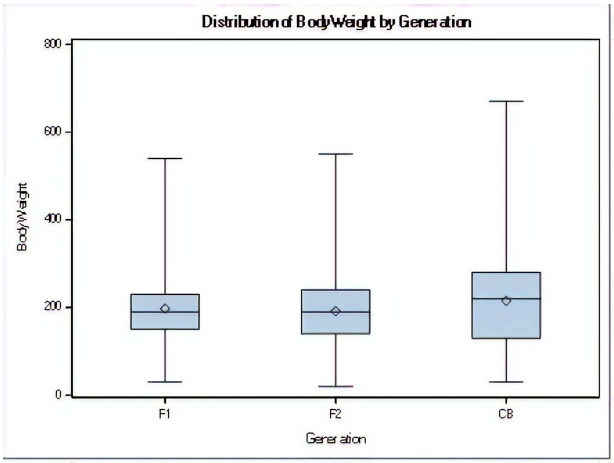


**Fig. 2b. Box Plot of body weight for $F_1$, $F_2$ generation and Cross Bred populaion**

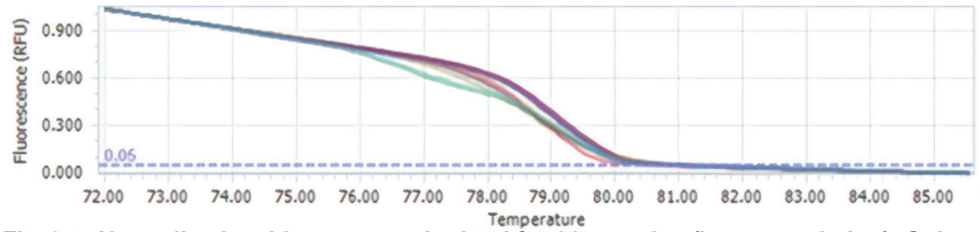


**Fig. 3a. Normalized melting curves obtained for 30 samples (base population). Colour codes suggest various HRM genotypes**

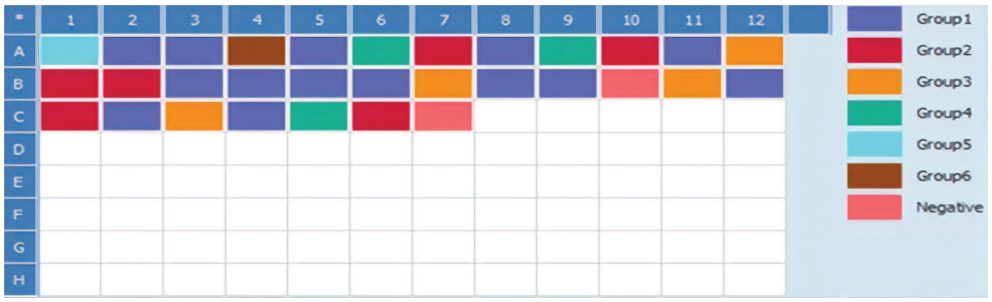


**Fig. 3b. The heat map shows distribution of samples under various groups (genotypes)**

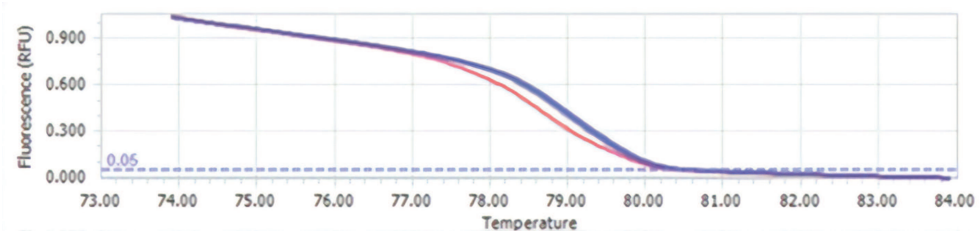


**Fig. 3c. Representative graph showing two classes of homozygotes with different alleles**

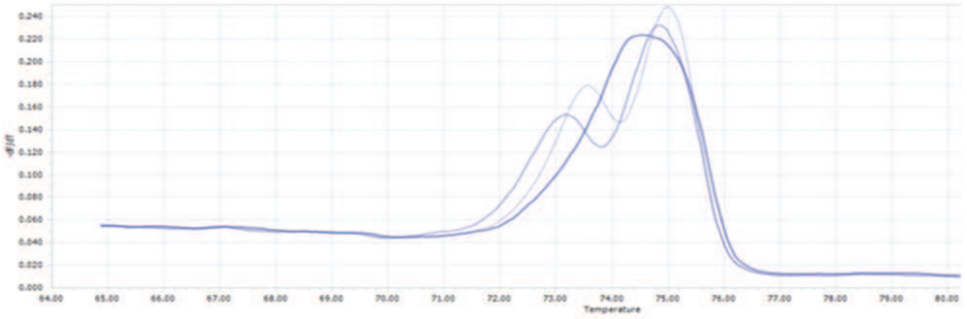

**Fig. 3d. Representative graph for differentiating homozygotes (one peak) and heterozygotes (two peaks)**

cross bred means for body weight and standard length were non-significant (Figs. 2a and 2b). The high selection differential and the high heritability for the traits may have resulted in increased genetic gain thus preventing the deleterious effect of inbreeding on growth traits.

Our results are in contrast to an earlier study reported in zebrafish (Mrakovcic and Haley 1979). They reported inbreeding depression in half sib mating and full sib matings for traits like fertility, survival to 30 days, and length at 30 days. Inbreeding affects the fitness (reproductive) traits more than the growth traits. Mrakovcic and Haley (1979) studied fitness traits as compared to the growth traits. Similarly estimates of inbreeding depression in body weight of brook trout (*Salvelinus fontinalis*) after one generation of brother-sister mating were found to be 27.7% at 7 months of age (Cooper 1961). However, it is also well known that, the rates of inbreeding depression vary among populations of the same species, and populations less sensitive to inbreeding depression are only selected as a potential source of outstanding inbred lines (Vianna et al. 1982; Lima et al. 1984). The inbreeding depression depends on both the level of dominance of a trait and on the inbreeding coefficient, the higher the level of dominance of a trait the higher the inbreeding depression (Falconer and MacKay 1996; San Vicente and Hallauer 1993; Benson and Hallauer 1994). Body weight and standard length in zebrafish may be governed by additive gene action rather than dominance and it could also be one of the reasons for no inbreeding depression

for selected traits in $F_2$ population.

### *HRM analysis*

The putative marker informativeness for 20 zebrafish microsatellite loci *viz*., number of alleles, PIC, heterozygosity, allelic diversity and tests for Hardy Weinberg Equilibrium was observed. The mean numbers of alleles per locus, mean expected heterozygosity, mean PIC for markers used was 9.050, 0.834 and 0.7876 respectively. The $F_{ST}$ value obtained for 30 samples belonging to three stocks (Assam, West Bengal and Tripura) is 0.223 which delineates structuring in zebrafish base population. The $F_{IS}$ value is 0.4031 and it indicates individual inbreeding coefficient present in the population for the selected markers. The normalized melting curve and heat map was used to genotype the samples (Figs. 3a and 3b). The shape of the melting curve depends on the GC content, length and sequence of the amplicon. The various curve shapes could be grouped into single inflection point graphs and graphs with two inflection points. Based on this, the genotype whether is homozygous or heterozygous can easily be distinguished by the shape of their curve (Figs. 5c to 5d). This analysis delineates selection of mating pairs that are heterozygous for a given markers and hence helps avoid the parent wherein the markers are fixed. Thus, specific DNA fingerprints can be generated for inbred lines. HRM analysis could serve as a rapid tool for genotyping and detection of putative alleles for microsatellites but with low discriminating power. However it has high efficiency in detection of SNPs. Distefano et al. (2012) successfully claimed the discriminating power of HRM with the traditional electrophoresis-based methods and provided a panel of primers for HRM genotyping in Citrus. Xanthopoulou et al. (2014) used HRM analysis, coupled with five microsatellite markers, integrated to facilitate molecular identification and characterization of main *O. europaea* cultivars collected from the National Olive Tree Germplasm Collection established in Chania, Greece. The five microsatellite loci used were highly informative and generated a unique melting curve profile for each of the 47 cultivars and for each microsatellite tested. HRM is an effective tool for microsatellites if they are priorly validated by sequencing and then standardised for HRM studies.

## Conclusion

A genetic repository named CZeBraG-Centre for Zebrafish Breeding and Genetic Research – A National Facility for Zebrafish Researchers is developed at ICAR-Central Institute of Fisheries Education, Mumbai. The study reports the first ongoing inbreeding program of zebrafish in India. The inbred zebrafish lines are being developed. The early genetic studies for two generations show that growth traits like standard length and body weight are highly heritable traits. There was no inbreeding depression for recorded growth traits. However early life history traits need to be incorporated for clear understanding of inbreeding depression. Genetic monitoring is conducted using microsatellite markers. The centre has commenced supply of pedigreed zebrafish for researchers. In future we can provide zebrafish for known inbred levels.

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## Authors' contribution

Conceptualization of research (MAP, GK); Designing of the experiments (MAP, AC); Contribution of experimental materials (MAP, GK); Execution of field/ lab experiments and data collection (MAP, AC); Analysis of data and interpretation (MAP, AC); Preparation of manuscript (MAP, AC, GK).

## Declaration

The authors declare no conflict of interest.

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