Techniques of detection of triploidy in tilapia essay

Design

Triploidy initiation which produces asepsis in Tilapia is an interesting option which has considerable possible in advanced aquaculture patterns (Penman et al. , 1987 ; Mair and Little, 1991 ; Mair, 1993) . Triploidization consequences in an add-on of excess set of chromosome by keeping of 2nd polar organic structure doing an addition in cell and atomic size (Graham et al. , 1985 ; Benfey, 1991 ; Stillwell and Benfey, 1996 ; Benfey, 1999 and Hyndman et al. , 2003) . Because of this excess set of chromosome, the cells of triploids maintain the nucleo-cytoplasmic ratio, hence, the cells of most of the variety meats (encephalon, retina, kidney, liver, testicle, ovaries) and tissues (blood, gristles, musculuss, epithelial tissue) are larger than those of their diploid opposite numbers (Benfey, 1999) .

However, in order to counterbalance the increased cell size and karyon, the variety meats and tissues of triploid persons have to cut down the figure of cells to keep its morphology similar to diploids (Maxime, 2008) . The exact scientific account, nevertheless, for this reduced cell figure is non yet understood. A assortment of direct sensing techniques have been used for distinguishing the normal and ploidy induced fishes viz. ; DNA staining and fluorescence quantification of Deoxyribonucleic acid with flow-cytometry micro-photometry, micro-densitometry, mechanical atom size distinction ; silver staining of nucleolar organiser parts and cytological karyotyping. However, each technique has its ain advantages and disadvantages, changing from proficient expertness, truth and expensive equipments, etc.

are required for such techniques. Direct finding method by cytological karyotyping is the most incontrovertible method of finding polyploidy among all these bing methods (Maxime, 2008) . The flow-cytometry is the fastest

and most accurate technique available among all known methods to find the ploidy in fishes (Maxime, 2008) . The other alternate indirect and easiest method to find ploidy is by look intoing the cells or nucleus size of red blood cells (Purdom, 1993 ; Tiwary et al. , 2004) .

A figure of surveies demonstrated that the red blood cell and atomic dimensions in triploid fish varied significantly from those of diploid fish. As the technique of erythrocyte measuring is simple, rapid and cheap, it is one of the most acceptable techniques. Other alternate indirect methods besides include coulter counter analysis, erythrocyte measuring of the chief axis length from computer-assisted image analysis and staining of red blood cell utilizing i¬, uorescent atomic discoloration (Maxime, 2008) . The atomic major axis measuring of red blood cell can readily and widely be used for placing triploids and besides tetraploids in Tilapia (Penman et al. , 1987) . Erythrocyte atomic measuring has been used to corroborate ploidy position of transgenic Tilapia, O. niloticus where the red blood cells of triploids is 1. 5 times larger than diploids (Razak et al.

, 1999) . Varadaraj and Pandian (1990) used RBC atomic volume for sensing of diploid and triploid fingerlings while bring forthing all female unfertile triploid in O. mossambicus. They recorded an addition in atomic size near to 1.

5 times greater than that of the diploid. Hussain et Al. (1995) used erythrocyte atomic major axis from the blood of grownup fishes of O. niloticus for know aparting triploid and diploid persons. A few consistent difference might happen among the haematological features of triploids

fishes which are viz. ; (1) larger red blood cells (2) increased hemoglobin content and (3) lesser red blood cell counts than erythrocyte counts of diploid (Benfey, 1999 and Cogswell et al.

, 2002) . This addition in cell size particularly of red blood cells helps in O transporting capacity from the external medium to the cell which might compromise the ability of triploids to use more O (Cal et al. , 2005) . The increased cellular volume in ruddy blood cells of triploids is geometrically associated with a lessening in the surface country to volume ratio (Cogswell et al.

, 2002) . The red blood cells of triploid persons exhibit curious morphological features with much higher frequences in comparing to diploids such as ; attenuated cell form and segmented karyon (Piercy, 2005) . A elaborate probe has been carried out by Pradeep (2010) to observe the triploids in ruddy intercrossed Tilapia. For his survey, he collected a entire figure of 60 larvae, 30 larvae produced by heat daze intervention (temperature 41o C) applied for continuance of 3.

5 proceedingss and 4 proceedingss after the fertilisation of eggs. A group of 30 larvae without using any daze intervention (command groups) were reared individually under indistinguishable H2O conditions in aquariums armored combat vehicles. The blood of actively swimming larvae (25 g in weight) from both groups was collected to analyze the red blood cell. Twenty specimens each from the control and the heat daze treated group of similar size were indiscriminately selected for the aggregation of blood. The designation of the ploidy degree of each fish utilizing good dispersed

metaphase phases was besides at the same time carried out to compare the consequences in both groups. For this, each fish was given a dosage of 0.

01 % colchicines at a rate of 1 ml/100 g administered with the aid of a subcutaneous syringe at the dorsal five, merely above to the sidelong line of the fish. This was followed by maintaining fishes inside an aerated fish tank armored combat vehicles (120 cubic decimeter) for 4 hours.

Differences in red blood cell measurings between diploid and triploid of Tilapia

Preparation of blood vilifications for red blood cell measurings

A fish at first was severed few millimetres anterior to the caudal peduncle to cut the caudal vena by a brace of crisp scissors for the aggregation of blood. A bead of blood was so taken from the cut part and placed on a clean microscopic slide and gently smeared utilizing a screen faux pas. Slides were allowed to dry for few seconds and so fixed in 95 % methyl alcohol. The slides were stained with Giemsa discoloration (10 %) for 20 proceedingss after proper drying and mounted by DPX and a screen faux pas, at the same time. Length and breadth of the cell and karyon were measured by a micron for 25 red blood cells from each group of fishes.

As red blood cells of Tilapia are ellipsoid in form, the cell and nucleus volumes are calculated by an equation ; V= 4/3Iˆab2 where a and B are the major and minor semi-axis of the cell and karyon (Uzunova, 2002) . The cytoplasmic volume was besides calculated by deducting nucleus volume from the average red blood cell cell volume whereas surface country of the

red blood cell cells and their karyon was calculated by an equation S= abIˆ/4 (Dorafshan et al. , 2008) . Photographs (1000X magnifications) of red blood cells collected from both the groups were taken for farther surveies.

Chromosome readying for sensing of ploidy degree

The same fish was used for chromosome readying to happen out the ploidy degree instantly after the aggregation of the blood for red blood cell survey (Sofy et al. , 2008) . The fishes were killed by pithing near to the encephalon, their kidney was removed and used for chromosome readying.

The kidney at first was washed decently in an isosmotic solution of NaCl (0. 7 %) to take extra blood and dust. NaCl was besides used to take tissues which were finely chopped utilizing a crisp blade. The shredded tissues from the petri dishes were so transferred to little plastic phials and so homogenized for a minute that was followed by their farther transportation to a bigger extractor tubing (15 milliliter capacity) . Tissues were so hypotonised with 8 milliliters KCl (0. 56 % kept at room temperature) which was added to each extractor tubing. Hypotonic intervention was given for a entire continuance of 40 proceedingss and so the solution changed two times at 15 proceedingss and 30 proceedingss of intervals. The attendant solution was so centrifuged at 100 tens g for 7 proceedingss and the supernatant removed carefully.

The tissues were instantly fixed in the same tubing with 8 milliliters cold Carnoy ‘ s solution (3: 1) for 30 proceedingss. The solution was once more centrifuged at 100 tens g for 10 proceedingss after the arrested development clip and the supernatant was removed and re-fixed in Carnoy ‘

s solution for 10 proceedingss at 4o C. The centrifugation and re-fixation procedure was repeated once more for 3rd clip after one hr before arrested development. After finishing the arrested development, the cell suspension and tissues were taken and placed on a clean microscopic slide. Tissues were chopped exhaustively to acquire white suspension utilizing a crisp scalpel. A bead of distilled H2O was put onto the tissue to forestall drying and for proper dissociation during chopping. Carnoy ‘ s solution (30 Aµl) was put onto the shredded suspension to ease proper spreading of cells on the slide. Cells were so spread utilizing the border of another microscopic slide.

Immediately, the slide with dispersed cells was warmed under the fire utilizing an intoxicant lamp until complete vaporization of liquid. The slide was so air dried for 10 to 15 proceedingss and subsequently rinsed in acetone solution to take the oil droplets. These slides were once more air dried for 10 to 15 proceedingss and so stained with newly prepared 10 % Giemsa discoloration (prepared in 0. 01M phosphate buffer ; pH= 7) for a period of 30 proceedingss. Slides were eventually rinsed in distilled H2O, air dried and mounted with DPX after 10 proceedingss of xylene wash. The metaphase spreads were photographed and figure of chromosome spreads was counted by detecting slides under 400X and 1000X (oil submergence) . The maximal figure of chromosome spreads every bit much as possible was counted for ploidy finding.

In control groups where no daze intervention was used, all persons were diploid (Pradeep, 2010) . However, in fishes where heat shocked intervention was given for triploid initiation, 20 out of 22 fishes were

triploids. The karyotyping analysis of diploid fishes showed the presence of 44 Numberss of chromosomes whereas 66 in triploid fishes. A important difference in cell and nucleus major axis, minor axis, volume, surface country and cytoplasmic volume was observed between diploid and triploid red blood cells. The different measurings of red blood cell of diploid and triploid ruddy intercrossed Tilapias are summarized in Tables 9. 1 & A ; 9. 2. In diploid, the average values of cell major axis, cell minor axis and cell surface country were ; 10.

43A±0. 51 ; 6. 69A±0. 36 Aµm and 54. 70A±4. 05 Aµm2, severally (Table 9. 1) . The average values of nucleus major axis, nucleus minor axis and nucleus surface country were ; 4.

47A±0. 34 ; 2. 50A±0. 25 Aµm and 9. 08A±1.

45 Aµm2, severally. Cell volume in diploid fishes was 245. 56A±29. 39 Aµm3 whereas nucleus volume 15. 89A±4. 25 Aµm3. The cytoplasmic volume in diploid was 229.

68A±26. 95 Aµm3 (Table 9. 2) . Similarly in triploid fishes, the average values of cell major axis, cell minor axis and cell surface country were ; 13.

32A±0. 37 ; 7. 46A±0. 44 Aµm and 77. 70A±6. 28 Aµm2, severally (Table 9. 1) . The average values for nucleus major axis, minor axis and surface country, were ; 5.

89A±0. 38, 2. 93A±0. 34 Aµm and 13. 66A±2.

29 Aµm2, severally. The cell volume in triploid fishes was 390. 67A±51. 69 Aµm3 whereas the nucleus volume was 26. 78A±6.

53 Aµm3. In triploids the cytoplasmic volume was 362. 81A±47. 60 Aµm3 (Table 9.

2) . The increases in cell major axis and minor axis were ; 27. 7 and 11. 5 % , severally, higher in the cells of triploid fishes as compared to cells of diploid fish. The spheroidal size of red blood cells in triploid fishes was comparatively larger as compared to the diploids. The addition in nucleus size in triploid was besides greater by 31. 7 % for the major axis as compared to minor axis by 17.

2 % . Similarly, the addition of nucleus cell surface and volume were ; 50. 4 and 68. 5 % , severally, as compared to cell surface country (42 %) and cell volume (59 %) in triploids. Cytoplasmic volume of the cell was increased by 57. 9 % in triploids as compared to diploid (Table 9.

2) . Table 9. 1: Cell and nucleus size and their combined parametric quantities of the diploid and triploid ruddy intercrossed Tilapia (average A± SD)[Reproduced from Pradeep (2010)]

Dimensions

Ploidy phase

Ratio [Diploid: triploid]

Increase

(%)

Significance

Diploid

(n= 20)

Triploid

(n= 20)

Cell major axis (Aμm)

10. 43 A± 0. 51

13. 32 A± 0.

37

1: 1. 27

27. 7

P & lt ; 0. 005

Cell minor axis (Aμm)

6. 69 A± 0. 36

7.

46 A± 0. 44

1: 1. 11

11. 5

P & lt ; 0. 0001

Cell surface country (Aµm2)

54. 70 A± 4.

05

77. 70 A± 6. 28

1: 1. 42

42. 0

P & lt ; 0. 0001

Nucleus major axis (Aµm)

4. 47 A± 0.

34

5. 89 A± 0. 38

1: 1. 31

31. 7

P & lt ; 0. 0001

Nucleus minor axis (Aµm)

2. 50 A± 0.

25

2. 93 A± 0. 34

1: 1. 17

17. 2

P & lt ; 0. 0001

Nucleus surface country (Aµm2)

9. 08 A± 1.

45

13. 66 A± 2. 29

1: 1.

50

50. 4

P & lt ; 0. 0001

(n= figure of specimens)Table 9. 2: Cell, karyon and cytoplasmic volume of the diploid and triploid ruddy loanblendTilapia (average A± SD)

[Reproduced from Pradeep (2010)]

Dimensions

(Aµm3)

Ploidy phase

Ratio [Diploid: triploid]

Increase

(%)

Significance

Diploid

(n= 20)

Triploid

(n= 20)

Cell volume

245. 56 A± 29. 39

390.

67 A± 51. 69

1: 1. 59

59.

0

P & lt ; 0. 0001

Nucleus Volume

15. 89 A± 4. 25

26.

78 A± 6. 53

1: 1. 68

68. 5

P & lt ; 0.

0001

Cytoplasmic Volume

229. 68 A± 26. 95

362. 81 A± 47. 60

1: 1. 57

57. 9

P & lt ; 0.

0001

(n= figure of specimens)The parametric quantities such as ; atomic and cytoplasimc volumes and atomic surface country though showed higher values in triploid as compared to the diploid fishes and it could be considered for finding the diploid and triploid fishes. But finding of these parametric quantities following the same technique is cumbrous and non easy to utilize. However, in O. mossambicus, Varadaraj and Pandian (1990) used RBC atomic volume for know aparting the diploid and triploid fingerlings where they found an addition in atomic size of 1. 5 times greater than that of the diploid.

In other survey conducted by Lincoln and Scott (1983) on rainbow trout, the atomic volume of RBC has been used for finding the ploidy in triploid. However, in triploid of ruddy intercrossed Tilapia, it has been 1. 68 times greater as observed by (Pradeep, 2010) .

The atomic volume ratio was 1: 1. 68 as compared to the values (1: 1. 86) obtained in Misgurnus anguillicaudatus (Gao et al. , 2007) . An addition in atomic volume by 87 % has been observed by Dorafshan et Al.

(2008) as compared to the theoretical expected 50 % addition. The atomic volume has been found to increase by 68. 5 % in triploids (Pradeep, 2010) . The erythrocyte volume of 45. 9 % has been reported about making close to the expected theoretical 50 % addition in sea bass (Dorafshan et al. , 2008) whereas in ruddy intercrossed tilapia the increase of red blood cell volume has been 59 % (Pradeep, 2010) .

The major and minor atomic axis were the other parametric quantities which showed comparatively the higher values in triploid as compared to the diploid fishes in ruddy intercrossed Tilapia (Pradeep, 2010) . As such these parametric quantities could easy be used to distinguish the diploid and triploid fishes. Penman et Al. (1987) has besides recommended atomic major axis measurings of red blood cell of Tilapia for the designation of triploid and tetraploid fishes. Razak et Al.

(1999) demonstrated the ploidy position of transgenic Tilapia, O. niloticus utilizing erythrocyte atomic major and minor axis measurings. In O. niloticus, the measuring of red blood cell atomic major axis has been found to be an appropriate option for placing the triploid and diploid persons as reported by Hussain et Al.

(1995) . In triploid of ruddy intercrossed Tilapia, the cellular major axis is 1. 27 times greater than the diploid (Pradeep, 2010) whereas in triploid sea bass it has been 1. 2-1.

3 times (Felip et al. , 1999) . Cell and atomic major axis, atomic and cell volume and cell minor axis were greatly different in diploid and triploid sea bass.

They suggested that the most appropriate parametric quantity to distinguish diploid and triploid is the major cell axis and cell volume. The cellular major axis in Caspian salmon has been reported 27 % higher than the minor axis (22 %) , therefore doing the cells ellipsoidal in form (Dorafshan et al. , 2008) . Similar consequence has been obtained in ruddy intercrossed Tilapia where the form of the cells is spheroidal in triploids (Pradeep, 2010) .

The cellular and atomic length and breadth have been found to be 25 and 20 % larger whereas atomic length and width 21 and 18 % greater in red blood cells of triploid than diploids in shortnose sturgeon (Beyea et al. , 2005) . The spheroidal size of red blood cells in triploid fishes is being comparatively larger as compared to the diploids. However, the morphology of red blood cells and their karyon in triploids of common carps has been found to alter from unit of ammunition to ellipsoidal in triploid as the major axis of the red blood cells enlarged more than the minor axis, well (Ueno, 1984) .

In creek trout, Salvelinus fontinalis, the addition in size of erythrocyte karyon with addition in figure of chromosome has been observed where the major axis is being recommended as the simplest method for ploidy designation (Woznicki and Kuzminski, 2002) . Cherfas et Al. (1994) besides showed a alteration in size and form of red blood cells and their karyon and used these parametric quantities in finding the ploidy degree in common carp (Cyprinus carpio) with a less than 4 % mistake. The red blood cells of triploid are 1. 5 times greater than diploids fishes.

The volume of karyon in triploids of ruddy intercrossed Tilapia has besides been found to increase by 68. 5 % than the diploids (Razak et al. , 1999) . The cytoplasmatic volume ratio in triploid ruddy intercrossed Tilapia is 1: 1. 57 whereas 1: 1. 48 in loach, Misgurnus anguillicaudatus (Gao et al. , 2007) .

The cytoplasmatic volume in triploid ruddy loanblend Tilapia has been 57. 9 % higher than the diploid fish (Pradeep, 2010) . Peruzzi et Al.

(2005) observed that the erythrocyte size of several indices including the addition in cytoplasmatic surface country (32 %) and nucleus (50 %) nevertheless, a considerable lessening in erythrocyte figure (34 %) in sea bass, Dicentrarchus labrax was observed. The surface country of the cell and karyon are ; 1. 42 and 1. 50 times higher severally in triploids of ruddy intercrossed Tilapia (Pradeep, 2010) whereas in triploid of common carps, these values ranged to 1. 44 and 1. 40 times higher (Ueno, 1984) .

However, there was no important consequence of ploidy on erythrocyte atomic minor axis (Svobodova et al. , 1998) .

Techniques of sensing of ploidy by karyotyping

The triploidy initiation survey, manner back twentieth century, was done in several fishes and crustaceans motivated the research workers to modify several conventional chromosome readying techniques. These modified techniques have largely focused on sensing of ploidy utilizing embryologic tissues (Kligerman and Bloom, 1977 ; Chourrout and Itskovich, 1983 ; Chourrout and Happe, 1986 ; Don and Avtalion, 1986 ; Hussain and McAndrew, 1994) . These research workers might hold taken embryologic tissues for ploidy sensing as the embryonic and larval phases are the most suited phases for chromosomal readyings where cells multiply quickly in soft tissues at these phases (Tan et al. , 2004) . Application of direct chromosome readyings is instead more desirable particularly for little larvae from which blood can non be collected. But the age or phase of larvae has a important influence on the quality of the clear metaphase phases (Chourrout et al.

, 1986) . Cytogenetic surveies in fish have valuable importance in evolutionary surveies, taxonomy, mutagenesis and in aquaculture industries particularly for ploidy finding and fish stock direction (Grey et al. , 1980 ; Chourrout et al. , 1986 ; Fenocchio et al. , 1988 ; Foresti et al. , 1993 ; Demirak and Unlu, 2001) . Promotion in teleost cytogenetical surveies has been followed by a assortment of karyotypic techniques including tissue civilizations (Roberts, 1964) , crushing of the testicle (Roberts, 1964 ; Ohno, 1965) , embryologic tissues or haematopoetic stuffs (Simon, 1963 ; Yamada, 1967) , smearing of gill epithelial tissue (Mcphail, 1966 ; Stewart, 1968) , kidney (Ojima, 1972 ; Arai, 1973 ; Ueno, 1977) and air drying techniques (Eicher, 1966 ; Fukuoda, 1972 ; Bertollo et al. , 1978 ; Thode et al.

, 1998) and colchicine intervention (Yamazaki, 1971) . Karyotyping or chromosome numeration is the analysis of chromosome figure and morphology of a species. Chromosomes can be obtained from any eucaryotic being whose cells are actively spliting.

With the advancement of genic surveies like chromosome set use, cytogenetic surveies have besides gained popularity in aquaculture reseraches well. Among the unreal polyploidy techniques, triploidization has been considered as the most successful engineering (Solar et al. , 1984) and successfully executed in several commercially of import species of fishes (Pandian et al. , 1998 ; Khan et al. , 2000) . In most of the old surveies, dropping method was used to scatter cells for karyotyping where tail part of larvae used for chromosomal readying. Among several techniques employed to fix fish chromosomes, air drying method of Evans et Al.

(1964) has been the most popular and widely adopted method. This method is the most recent one and used efficaciously for fixing carnal chromosomes whereas the squash technique is the oldest and the most widely used method for distributing and flattening metaphase chromosomes (Denton, 1973) . Although these methods gave some consequences, a big part of cells has been found to be lost during the experiment as a consequence of dropping the cell from a tallness. More over this method requires more proficient accomplishment to drop cells precisely onto the top of a preheated slide. Some research workers have besides attempted to drop cells from a tallness onto the frozen slides for fixing the chromosome spreads (Ojima et al. , 1964 ; Ida et al.

, 1978 ; Lamatsch et al. , 1998) . Researchers even tried with H2O vapor from a H2O bath for heating the slides and distributing cells (Henegariu et al. , 2001) . Chourrout and Happe (1986) reported that the conventional techniques of chromosome readying by air drying technique after colchicine injection in immature fishes resulted in unequal metaphase spreads (MacPhail and Jones, 1966 ; Kligerman and Bloom, 1977) . Observation of sex chromosomes from cytological readyings allows allowing an accurate proofreading distinction which is an accurate method for straight distinguishing the diploid or triploid persons with 2 and 3 sex chromosomes (Moreira-Filho et al. , 1993 ; Devlin and Nagahama, 2002 ; Molina and Galetti, 2007 ; Vasconcelos, 2009) . Karyological surveies have given valuable information on the figure, size and morphology of chromosomes which is indispensable for polyploidy use in fish (Khan et al.

, 2000) . Therefore, cytogenetic application or karyotyping has been considered as a critical tool for honing the stock sweetening techniques such as chromosomal use, hybridisation and other related familial technology techniques (Tan et al. , 2004) .

Preparation of chromosome spreads

In a survey carried out by Pradeep (2010) karyotyping, tissues from one twenty-four hours old larvae of unnaturally bred ruddy intercrossed Tilapia were collected for chromosomal spreads. The colchicines concentration was ab initio optimized to acquire the maximal metaphase spreads. For this series of test experiment were conducted.

For each experiment extra test with 20 larvae per batch was considered. First test was performed utilizing larvae from one batch whereas the 2nd on another batch of larvae. Three different concentrations of colchicine i. e. 0. 005, 0. 01 and 0.

1 % of 20 milliliters were taken in three separate petri dishes. Twenty larvae (one twenty-four hours old) for each concentration were taken and immersed into the colchicine solution. For each concentration, the continuance of the colchicine intervention was besides altered for 2, 4-6 and 10 hours. In all test experiments, newly prepared colchicine was used and samples were kept undisturbed at the room temperature. After coveted continuances of 2, 4-6 and 10 hours, all larvae from different batches of colchicine interventions were taken out from the petri dishes and transferred into an isosmotic solution of 0.

7 % chilled Na chloride individually, to anesthetize larvae. The yolk pouch and dust of larvae were carefully removed utilizing a crisp scalpel. In continuance an effort was farther made to optimise the continuance of hypotonization. For this the whole organic structure was hypotonized in 0. 56 % K chloride (0.

075 M) for continuances of 20, 30, 40 and 50 proceedingss at the room temperature to happen out the most effectual hypotonic intervention. Similarly, in order to happen out the most appropriate ratio for Carnoy ‘ s solution, 3 different ratios of methyl alcohol: acetic acid (2: 1, 3: 1 and 4: 1) of Carnoy ‘ s fixative was prepared and testified. The complete organic structure of the larvae was so fixed in a little glass vial incorporating 15 milliliter of newly prepared Carnoy ‘ s solution with different ratio and kept individually at 4o C.

The fixative was ab initio changed twice at 20 proceedingss intervals in the same phial. After this these larvae were stored with fixative at 4o C for a minimal continuance of 6 hours inside the same glass phial. Slides for chromosome readying were cleaned exhaustively before their usage. Slides and cover faux pass were kept in 95 % ethanol overnight and so rinsed with distilled H2O (Yu et al. , 1981 ; Sofy et al.

, 2008) followed by swobing of the slides by soft tissue paper. After arrested development, whole organic structure of the larvae was taken out from the vial and on one slide one larva was taken for chopping. A bead of distilled H2O was put on the larva to forestall drying and for the proper dissociation of embryologic cells. Choping method as suggested by Yamazaki et Al.

(1981) was so followed. The effectivity of chopping method was besides checked utilizing both distilled H2O and 50 % acetic acid as described by earlier workers.

The larva was so chopped exhaustively utilizing a crisp scalpel to acquire white suspension. A 30 Aµl of Carnoy ‘ s solution was put onto the shredded suspension to ease proper spreading of the cells throughout the slide. Cells were so spread utilizing one side of another microscopic slide. Immediately, the slide with dispersed cells was warmed under the fire utilizing an intoxicant lamp until the liquid evaporated copletely. Slides were so air dried for 10 to 15 proceedingss and subsequently rinsed in acetone solution to take the oil droplets. All slides were once more air dried for 10 to 15 proceedingss and so stained with newly prepared 10 % Giemsa discoloration (prepared in 0. 01M phosphate buffer of pH= 7) .

Different concentrations of Giemsa discoloration (5, 10 and 20 %) under altered timing of 10, 20 and 40 proceedingss were examined and compared. After staining, slides were dipped in xylene for 10 proceedingss. Following the xylene wash, slides were later rinsed in distilled H2O, air dried and mounted with DPX. The metaphase spreads were photographed and figure of chromosome spreads were counted under 400X and 1000X (oil submergence) . As many chromosome spreads as possible were counted on each slide and compared to choose the most appropriate parametric quantities for acquiring the best and maximal chromosome spreads.

However, chromosome spreads which were either excessively spread or clumped, excluded during the numeration. The batch demoing highest figure

of good metaphase spreads in all test experiments was selected for farther experiment. The appropriate concentration of colchicine (0. 01 %) , continuance of colchicine intervention (4-6 hours) , hypotonic interventions (for 40 minute) , Carnoy ‘ s solution (methyl alcohol: acetic acid ratio 3: 1) for arrested development and concentration of Giemsa (10 %) for 20 proceedingss continuance ; were considered for farther experiments by Pradeep (2010) . Consequences of this improved method showed that good chromosome spreads could be made from the whole organic structure of ruddy intercrossed Tilapia.

The minimal and maximal figure of chromosome spreads were ; 13 and 25 for the diploid severally whereas 11 and 21 for triploid severally on one slide. In the present experiment the whole organic structure was taken because of the fact that at embryologic phase of the fish, a really small crystalline tissue was available for doing the spread. Slides with diploid and triploid embryonic larvae showed a high per centum of chromosomal spreads runing from 95-100 % in all the three experimental efforts utilizing different batches of the larvae. Second, the improved technique eliminated the drooping of cell suspension onto pre-heated or frozen slides. Alternatively, the chopping method besides avoids the dropping of cell suspension where all stairss of readyings are carried out merely on one slide within a shorter continuance. For karyotypic analysis, every individual measure including the readying of tissues and slides are critically of import for obtaining big figure of good dispersed metaphases. By and large, a cell civilization population has a mixture of cells at all phases of the cell division rhythms at any given clip.

However, chromosome readyings could merely be possible when this cell civilization population has adequate mitotic cells.

For enriching these mitotic cells, colchicine drugs are used which allowed arresting of chromosome division at a metaphase phase of the cell division. Optimum concentration of colchicine and the continuance of interventions are critically of import for acquiring better consequences (Pradeep, 2010) . Thus the initial measure in the technique is colchicine intervention of the being for collaring the cell division. The optimal colchicine concentration for ruddy intercrossed Tilapia fish larvae has been determined as 0. 01 % for 4-6 hours (Pradeep, 2010) . Hussain et Al. (1994) has already reported that inadequate or over concentration of both colchicine interventions can take to many un-burst cells with uncountable and over lapping chromosomes in the slide readying.

Pradeep (2010) besides showed many un-burst cells that resulted in awkwardness of cells rather frequently during altering in concentrations of colchicine (0. 005 % and 0. 1 %) .

Duration of 2 and 10 hr interventions showed limited chromosome spreads and more gawky cells. Colchicines stored in a icebox at a temperature of 4o C has non been found every bit effectual as newly prepared colchicines solution. Hypotonic intervention is an of import and important factor in bettering the chromosome spreads. This intervention helps in remotion of lipoids and denatures proteins. It besides allows the puffiness of the cell which facilitates cell break and the scattering of chromosomes when the cell contents are spread on slides. Ida et Al. (1974) reported that K chloride

facilitated the best chromosome spreads as compared to sodium citrate and distilled H2O.

The hypotonic intervention with K chloride has been standardized for 40 proceedingss where a comparing with intervention times for 20, 30, 40 and 50 proceedingss are used in ruddy intercrossed Tilapia. A clip of below 40 proceedingss resulted in more sub-burst cells whereas ; chromosomes are found overlapping for 50 proceedingss. Chourrout and Happe (1986) reported that the chromosome spreading was deficient with 0. 56 % KCL for hypotonic intervention in rainbow trout at the lower temperature. However, it showed somewhat better consequences by executing the experiment at ambient temperature.

Harmonizing to the same writer, trisodium citrate as hypotonic intervention gives important betterment in chromosome spreading. However, 0. 56 % KCL intervention for hypotonization with appropriate continuance at room temperature has been found to give better consequences (Pradeep, 2010) . A fixative solution of Carnoy ‘ s at a ratio of 3: 1 has been most effectual as compared to both 2: 1 (Ida et al. , 1974) and 4: 1 ratio (Hussain et al.

, 1994) . The Carnoy ‘ s fixative allows in continuing the internal construction of the cells for better staining of the chromosome (Comings, 1978) . The tissues can be stored in this Carnoy ‘ s solution for a longer continuance of more than a month (Pradeep, 2010) . In the technique improvised by Pradeep (2010) for ruddy intercrossed Tilapia, the stairss followed by Klingerman and Bloom (1977) and Hussain et Al. (1994) of

maceration and dissociation of cells with 50 % and 60 % acetic acid severally were avoided.

Distilled H2O (2-4 Aµl) has been used for forestalling the cells from drying when maintain onto slides for chopping (Pradeep, 2010) . In the modified technique, different continuances of staining along with different concentrations of Giemsa discoloration were besides tried. A concentration of 5 % Giemsa discoloration for its intervention of 20 proceedingss as described by Bayat and Woznicki (2006) was non really effectual whereas, at a concentration of 20 % as suggested by Don and Avtalion (1986) , numbering of the chromosome was hard. The altered timing and concentrations of Giemsa discoloration affected significantly the visibleness and brightness of the spreads on slides. A concentration of 10 % Giemsa discoloration prepared in 0. 01 M phosphate buffer of pH 7 for 20 proceedingss continuance as described by Hussain et Al. (1994) was besides tried in ruddy intercrossed Tilapia obtained clear images.