CHAPTER V

SUMMARY AND RECOMMENDATION

Summary

1. Hatching rate of embryos which were microinjected at one-cell, two-cell and four-cell stage, were 25.91 %, 29.71 % and 30.94 %, respectively, showing no significant differences ($P>0.05$), but when compared with the control (40.67 %, uninjected embryos), all injected embryos showed significant lower hatching rate ($P<0.05$).

2. The physical injury of microinjection technique was a major factor affecting hatching rate of microinjected eggs.

3. Microinjection at earlier embryonic development stage could be easier performed than the later stage.

4. Microinjection manipulation at temperature 25 ^{0}C could delay embryonic development for prolonged time of microinjection performance.

5. Survival rate at one month of fry which were derived from microinjection egg at one-cell, two-cell, four-cell and control were

52.02 %, 46.67 %, 35.51 % and 53.61 %, respectively, showing no significant differences.

6. By using PCR methods combined with hybridization for detection the introduced gene in fish genome of blood sample, the integration rate of introduced gene in transgenic fish, which was derived from one-cell, two-cell and four-cell embryos, was 5.05 %, 6.45 % and 8.5 %, respectively.

7. This study found the mosaic transgenic among various tissues of transgenic fish in all cell-stage development microinjection. By microinjection at one-cell stage, more tissues were accomplished the integration of introduced gene than microinjection at later stages.

8. Growth rate of fish which were derived from one-cell and two-cell microinjected embryos, was higher than those of control. Growth rate of fish which were derived from four-cell microinjected embryo was similar to that of the control. However, growth rate of fish derived from only one-cell embryo was significantly higher than that of the control ($P<0.05$)

9. Appropriate embryonic stages for production of transgenic yellow walking catfish by microinjection were one-cell stage and two-cell stage.

10. Production of transgenic fish by microinjection method would be a valuable tool for genetic improving of growth in the yellow walking catfish in the future.

Recommendations

1. Both high quality matured male and female yellow walking catfish should be used for production of the high fertilization rate in order to improve hatching rate or survival rate of control (uninjected embryos) and microinjected embryos.

2. In order to increase the efficiency of transgenic yellow walking catfish production, microinjection of the introduce gene should be carried out at one-cell stage and two-cell stage.

3. Improving the optimal condition of PCR for detection a very little introduced gene should additionally investigate some factors such as concentration of magnesium ion, Deoxynucleotide (dNTP), taq polymearase and primer, buffer pH PCR cycling number, temperature and time of denaturation, annealing, and extension of each cycle.

4. Number of fish that were sacrificed for detection the mosaic transgenic should be increased in order to evaluate the mosaic characters.

5. Southern blot hybridization should be used for studying the integration characters, integration copies, in fish genome.

6. For easy detection, the transgenic fish, marker gene might be used to fuse with the interesting gene. Then integration and expression could be detected by the product of marker gene.

7. Transmission of the transgenic yellow walking catfish should be continually investigated by breeding with non transgenic yellow walking to study efficiency of genetic improvement by transgenic fish production.