Chapter V

Conclusion
5. CONCLUSIONS

The uses of viral resistant shrimp lines is an important preventive measure in aquaculture management strategies aimed to reduce the economic damage due to viral diseases. The first feasible step towards immediate control is to use disease resistant broodstocks and to fulfill this goal one of the strategic scheme is to use DNA based markers. Aquaculture of *Penaeus monodon* is one of the economically important sectors for many countries including India. Study of shrimp genome is important in shrimp health management yet, their genomic structure is barely known to us. Sustainable development of this industry could greatly benefit from progress in our fundamental knowledge of genetics, genomics and molecular immunology of shrimp. Significant advances in the genome information of several important aquaculture species are already well known due to the development of different molecular technologies capable of efficiently sequencing the DNA but in shrimp, little is known about the gene function. Since, SPR domesticated broodstock of *P. monodon* is not yet readily available, and most of the countries depend on wild unselected broodstocks for shrimp aquaculture, so it is necessary to minimize the entry of pathogens as well as use of disease resistant seeds into the culture shrimp production systems. In the present investigation, one 457 bp RAPD SCAR marker and three microsatellite DNA markers of sizes 773 bp, 299 & 262 bp is developed.

Alongside, as furtherance exertion of the previously developed 71 bp microsatellite DNA marker, the practical utility of this DNA marker and newly developed DNA markers also is confirmed by WSSV challenge experiment. Post challenge results showed that the mortality was significantly higher among disease susceptible shrimps, and WSSV propagation was also very high among them. Quantitative real-time PCR analysis of WSSV propagation at 72 hrs post challenge, suggested that the viral load was $1.21 \times 10^3$ fold higher in disease susceptible as compared to disease resistant populations those were identified using 71 bp microsatellite DNA marker. In case of the shrimps samples, which were pre-identified by the developed 457 bp SCAR marker the quantitative real time PCR data showed that the virus copy at 72hr of challenge experiment was $2.25 \times 10^3$ fold higher in WSSV exposed disease susceptible shrimps. Additively, the challenge experiment data for the newly developed microsatellite DNA markers (773 bp, 299 bp and 262 bp) showed that the survivability among disease resistant shrimps were significantly higher as
compared to the disease susceptible shrimps selected using 773 bp microsatellite DNA marker but it was more higher in case of disease resistant shrimps which were identified using 299 bp and 262 bp marker. This may be due to the non-additive effect or polygenic effect of the disease resistance trait. Consequently quantitative real-time PCR data showed that the virus copy at 72hr of challenge experiment was \( \sim 1.4 \times 10^3 \) fold higher using 773 bp and \( \sim 1.4 \times 10^3 \) fold higher using 299 bp and 262 bp marker both in disease susceptible shrimps. In conclusion, the result presented here is the preliminary work to develop disease resistant DNA marker in *P. monodon*, although it is necessary to study further to know whether this disease resistant marker is a WSSV disease resistant quantitative trait locus (QTL) region. The deduced sequence information of the developed DNA markers in *P. monodon* will be helpful in identification of probable genes involved in disease resistance or disease susceptibility which will give many information about the mechanism of disease resistance or susceptibility in future.

In the present investigation, disease resistant prevalence was also investigated among the wild captured *P. monodon* from nine distinct geographic locations using the developed DNA markers. Results for disease resistant prevalence using 71 bp microsatellite DNA marker suggested that among the wild caught samples of *P. monodon*, the disease resistance from Chilika, Orissa; Visakhapatnam, Andhra Pradesh and Port Blair, Andaman Sea was the highest among the five places along the East coast of India. The assessment of disease resistance using the newly developed 773 bp, 299 bp, and 262 bp microsatellite DNA showed Vasco-da-gama, Goa; Chilika, Orissa; Visakhapatnam, Andhra Pradesh, and Veraval, Gujarat had the higher disease resistant prevalence. Combining the result obtained from all DNA markers showed wild broodstock collection from Port Blair, Andaman Sea; Visakhapatnam, Andhra Pradesh; Chilika, Orissa along the East coast and Vasco-da-gama, Goa and Veraval, Gujarat along the West coast will be more useful to get disease free as well as disease resistant seeds to prevent vertical and horizontal transmission of WSSV. These study results give an easy, cost-effective and immediate solution to the farmers facing problems due to the viral disease in much lesser time.

It is seen that disease resistant populations are sparingly distributed in the marine environment. If the genotype of the shrimps is of the susceptible type then with the onset of favorable conditions, the shrimps will get become infected with WSSV.
Therefore, the selection of these disease resistant broodstocks and breed those in an environmentally controlled place like in hatchery will eventually increase genetic uniformity and thus decrease disease risk. The increasing need of this species for human consumption can be fulfilled only from aquaculture. Thus, enrichment of disease resistant broodstock generation and consequently cultivation of seeds developed from them could be a path breaking step towards disease prevention. Concerns in the field of genetic monitoring of shrimp farming have increasingly become more proficient in the prevention of large-scale production failures. Data on WSSV and disease-resistant prevalence in wild *P. monodon* would be important to develop strategies for shrimp health management.