ICAR-CIFA

A PCR Based Detection Kit for Rohu X Catla Hybrid

Sustainability of aquaculture industry largely depends on the availability of the quality seeds. Inadequate supply of quality seed is often suggested as a major constraint for aquaculture in many parts of the world. The issue of quality comes to the attention of producers only after a certain period of time when performance indicators (e.g. growth, production, survival and disease) consistently manifest seed quality. Indian major carps (IMCs) comprising of rohu *(Labeo rohita)*, catla *(Catla catla)* and mrigal *(Cirrhinus mrigala)*, owing to their fast growth and taste enjoy a prime position in the Indian aquaculture scenario. Taking the advantage of seed demand of these two species, many hatchery owners and seed producers supply hybrid seed (catla x rohu or rohu x catla) of these two species in the name of pure rohu or catla during the young stages. These hybrids cannot be easily differentiated from each other morphologically at early stages of development e.g. hatchling and early fry stages.

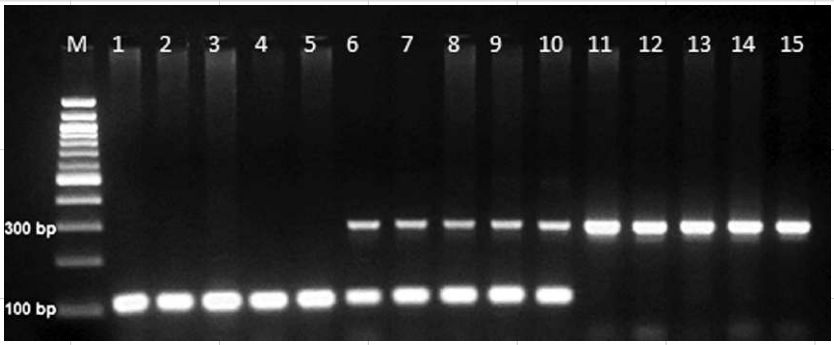
Hybridization technique was used by aquaculturists in the hope of producing aquatic organisms with specific desirable traits or general improvement in performance. Natural occurrence of both interspecific and intergeneric hybrids of Indian major carps has been reported mostly from reservoirs and other natural ecosystems. Several interspecific and intergeneric hybrids of Indian major carps: *Catla catla*, *Labeo rohita, Cirrhinus mrigala* and *Labeo calbasu* and those of Indian major carps with exotic carps viz. common carp and silver carp have been artificially produced through hypophysation. These hybrids were not popular due to poor survival among some undesired traits exhibited by them. Further, many species are jeopardized by hybridization and genetic introgression, and these are potential threat to the diversity of freshwater fish particularly when the hybrids are fertile. They may also compete in several ways with the native parental lineages.

With the rapid expansion of aquaculture, entire seeds of IMCs used for culture are produced at private rearing facilities, and fry are subsequently distributed to fish farms. These profit driven hatchery owners set their target on quantity of spawn rather than quality. Usually, small number of broodfish and practice of mixed spawning to minimize cost of production results in poor quality fish seed with hybrids. In a survey conducted by ICAR-CIFA it has been found that on an average 22.8% of the total hatchery produced seeds are hybrids. Therefore, genetic monitoring of hatchery stocks on regular basis is required to maintain quality of fish seeds.

Currently, several genetic markers have been developed for different purposes to be in aquaculture programs. PCR-RFLP and multiplex-PCR are considered fast, simple and inexpensive, but they have rarely been used in the characterization of hybrids or in aquaculture in general. These techniques are important tools in species identification, especially in studies related to the biological conservation and forensic identification.

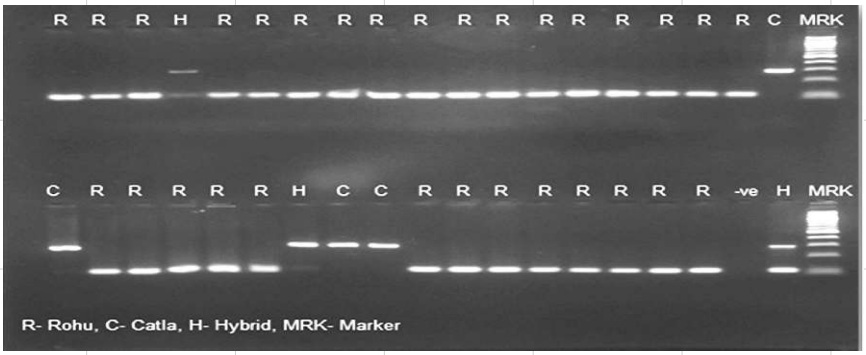
In this background a PCR based identification kit has been developed which can identify in a hybrid in just two steps with genomic DNA as starting material. This is a specific kit designed for the identification of *Labeo rohita (rohu), Catla catla (catla)* and their hybrid in the early life stages using a house keeping nuclear gene (Figure 1 & 2).This technology would really be of great use for those producers and buyers for screening of quality seeds and moreover it would be a stepping stone for seed certification programs for both government and private hatcheries.

Figure 1. PCR amplification for the presence of hybrids using actin species specific primers



Lane M: 100 base pairs (bp) marker, Lane 1-5, Rohu (100 bp); 6-10, Hybrid (both 100 and 300 bp); 10-11, Catla (300 bp).

Figure 2. PCR amplification for the presence of hybrids with -actin species specific primers collected from different carp hatcheries.



ADVANTAGES& UTILITY OF THE KIT :

- PCR and agarose gel based detection kit
- No sequencing is required
- Takes only 4-6 h to get the results
- Highly sensitive and specific for rohu X catla hybrids and reciprocal crosses
- Useful for screening of hatcheries for genetic contamination
- Potential for seed certification hatchery accreditation
- An essential tool for Government/private agencies to ensure purity of seed

CONTENTS OF THE KIT:

1. Species specific primers
2. Universal primer
3. dNTP mix (2.5mM each)
4. 10X Taq DNA buffer
5. Taq DNA Polymerase 3U/µl
6. Positive control DNA 100ng
7. Nuclease free water
8. 100bp ladder.

PRICE OF THE KIT IS Rs.20/- PER SAMPLE ONLY

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