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Interferon-regulatory factors, IRF3 and IRF7 in Asian seabass, *Lates calcarifer*: Characterization, ontogeny and transcriptional modulation upon challenge with nervous necrosis virus

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ABSTRACT

Interferon regulatory factor (IRF) 3 and IRF7 are key regulators of type I interferon (IFN) gene expression for the antiviral immune response. In the present study, interferon regulatory factor 3 and 7 from Asian seabass, namely AsIRF3 and AsIRF7 were cloned and characterized. The full-length cDNA sequence of IRF3 and IRF7 consisted of 2965 and 2343 bp respectively. AsIRF3 and AsIRF7 were true orthologes of vertebrate IRF3/7 and showed similar domain organization, with an N-terminal DBD which consisted five tryptophan residues in IRF3 and four in IRF7, a C-terminal IRF3 domain and a serine rich region. Both IRF3 and 7 constitutively expressed during the ontogenesis and in all tissues of healthy fish. The expression of both genes was up-regulated following NNV challenge with obvious transcript abundance in brain heart and kidney. Ectopic expression of AsIRF3 and AsIRF7 displayed activation of ISRE/NF-κB promoters and modulation of interferon, ISGs and pro-inflammatory cytokine gene expression. These observations indicated that IRF3 and IRF7 play an important role in Asian seabass's antiviral defense and the RIG-IRF-IFN axis is conserved in the species.

1. Introduction

Interferons (IFN) are a group of multifunctional polypeptides called cytokines that they can activate immune cells and also directly inhibit viral replication. Interferons are of two types, type-1 IFN, (which includes IFN-α and IFN-β) and type-2 IFN (IFN-γ). A variety of infected cells produce type-1 IFN [1] whereas type-2 interferon is produced by activated T cells and natural killer cells [2]. The transcription factors of the IRF family play a critical role in the activation of IFN genes involved in host immune responses [3]. IRF has a role in antiviral defense, immune response and cell growth regulation by acting as transcriptional mediators of type-1 interferon and IFN stimulated genes [4]. To date, at least 11 IRF members (IRF1 to IRF11) have been identified in vertebrates [5]. Each IRF is characterized by the presence of five conserved tryptophan repeats three of which contact DNA, recognizing the AAN NGAAA sequence in a well-conserved N-terminal DNA-binding domain (DBD) of ~120 amino [6]. The DNA binding domain of IRFs bears a resemblance to the DBD of Myb transcription factors [7]. The carboxy terminus of all IRFs except IRF1 and 2, form transcriptional complexes due to interaction with proteins by virtue of an IRF association domain [8].

IRF3, IRF5, and IRF7 among the IRF family has an important role in the inducing expression of type-I IFN genes in infected cells and strongly synergize with the virus-inducible expression of IFN-α and IFN-β promoters [4]. IRF3 and IRF7 have greatest structural homology and are important in RIG-1/MDA5-mediated type-1 interferon pathway. Recognition of viral nucleic acids in most of the nucleated cells is because of TLR3 and RNA helicases RIG-1 and MDA-5 [9]. Phosphorylation of specific serine residues in C-terminal (regulatory) regions upon viral infection causes translocation of IRF3 and NF κB from cytosol to nucleus [10]. IRF3 and NF-κB upon translocation into the nucleus initiates transcription causing activation of classical JK-STAT pathway and interferon stimulating gene factor (ISGF)- 3 [11]. Following virus infection, IRF3 causes induction of type-1 and it is reported that mice deficient for IRF3 are more vulnerable to viral infection because of drastic reduction in IFN production [12]. The role of IRF7 was demonstrated in vertebrates and it has been reported that IRF7 controls important IFN responses like the systemic production of IFN innate immunity and the local action of IFN from plasmacytoid dendritic cells in adaptive immunity [13].

Over the last decade, a lot of research has been done to understand the antiviral responses of fish. Fish IRFs are reported to play an

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important role in antiviral defense like the induction of type 1 IFN, roles in the functional and developmental processes occurring in fish lymphomyeloid tissue, modulation of inflammatory genes and suppression of viral replication [14–16]. Asian seabass is considered as a candidate mariculture species in India. But the catastrophic loss in the larval development stages caused by the nervous necrosis virus (NNV) is a limiting factor in its production. In the present study we characterized the IRF3 and IRF7 from Asian seabass and evaluated its functional characteristic and ontogeny.

2. Materials and methods

2.1. Fish maintenance and sample collection

Healthy Asian seabass fingerlings of size 2.5–3.0 cm were procured from Seabass hatchery project (Rajiv Gandhi Centre for Aquaculture) Tamil Nadu, India. The animals were acclimatized to the laboratory conditions prior to commencement of study. The fishes were anesthetized and experimental tissues including kidney, spleen, hindgut, liver, heart, muscle, brain and gills were collected for RNA extraction.

Embryonic and larval developmental stages were collected from the Seabass hatchery project. Seven different embryonic developmental stages: i.e., unfertilized egg (Unf), fertilized egg, 138-cell stage, 3 h post-spawning (3 hps), blastula (5 hps), gastrula (7 hps), Neurula (9 hps) and early embryo (12 hps), and seven different larval developmental stages: viz., 1, 3, 5, and 7 day post-hatching were sourced. Embryonic and larval developmental stages were collected from three different larval rearing units and three separate pooled samples were used for RNA extraction.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from different tissues and developmental stages using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. First-strand cDNA was synthesized from DNase treated total RNA using Super- Script III First Strand synthesis system (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol.

2.3. Molecular cloning and sequencing of Asian seabass IRF3 and IRF 7

Degenerate primers IRF3-F1 and IRF3-R1 and IRF7-F1 and IRF7-R1 were designed based on the conserved amino acid sequence for IRF3 and IRF7 cDNA amplification. All PCR products were gel-purified, cloned into pTZ57 R/T vector (Thermo scientific, Vilnius County, Lithuania) and sequenced. Rapid amplification of cDNA ends (RACE) was performed to obtain 5′UTR (untranslated region) and 3′UTR of IRF3 and IRF7 cDNA by using SMARTer™ RACE kit (Clontech, Terra Bella Avenue, Mountain View, CA, USA). The full-length cDNA of IRF3 and IRF7 was cloned into pTZ57 R/T (Thermo Scientific, Vilnius County, Lithuania) and sequenced at M/s Bioserve Biotechnologies Pvt. Ltd., India.

2.4. Genomic and putative protein structure analysis and phylogenetic tree construction

The nucleotide and deduced amino acid sequences of IRF3 and IRF7 cDNA from Asian seabass were analyzed using BioEdit 7.0.1 and Expasy search program (http://au.expasy.org/tools/). The sequences of IRF3 and IRF7 from different species were compared by the NCBI BLAST Search Program. Genomic structures were analyzed by using specialized Blast program (http://blast.ncbi.nlm.nih.gov/Blast). A Multalin alignment was made with similar protein sequences after BLASTp analysis with different organisms in the evolution-scale, and then the Neighbor-joining tree was performed with MEGA software version 7.0 [17].

2.5. NNV challenge

For in vivo challenge study, 54 fishes were given immersion challenge with 10 mL of 2.9×10^8 $TCID_{50}$/mL dose of cell culture derived NNV (RGNNV genotype) in 10 L of water (5‰ salinity, pH 7.6 ± 0.5) for 2 h and the control groups (n = 54) were exposed to HBSS. After the challenge, fishes were divided into 3 groups of 18 animals each and maintained in clean water. Nine animals each (n = 3 from each tank) from infected and control groups were collected at six time points (3 h, 6 h, 12 h, 24 h, 48 h, and 72 h) post challenge. Tissues (Brain, gill, heart, spleen, liver, and kidney) were collected aseptically from the sampled fishes and stored in RNAlater (Sigma, USA) for further analysis. Tissues collected from three animals were pooled and three of such pools were analyzed at each time point.

2.6. Real-time PCR analysis of IRF3 and IRF7 mRNA expression

Real-time PCR (qPCR) was carried out in a Roche LC96 LightcyclerTM (Roche, Switzerland) using a 96 well plate layout. The following volumes were used per reaction (total volume 10ul): 5 µl of 2X SybrGreen reaction mix (Invitrogen, USA), 0.2 µl each of 10 pmol/µl forward and reverse primers, 1 µl of 100 ng cDNA and nuclease free water to make up the volume. The thermal cycling conditions were: 1 cycle of 10 min at 95 °C to activate the polymerase, 40 cycles of 10 s at 95 °C and 1 min at 60 °C for amplification. Dissociation curve analysis was carried out to verify the absence of primer dimers and/or non-specific PCR products. Expression of genes of interest was normalized to the mean of EF1α as the house keeping gene [18]. Each sample was analyzed in triplicate to certify the repetitiveness and credibility of experimental results. To maintain consistency, the baseline was set automatically by the software. The comparative CT method ($2^{-\Delta\Delta CT}$ method) [19] was used to analyze the expression level of all the genes tested. Primers used were listed in Table 1.

2.7. ISRE and NFkB dual luciferase assay

The full length ORF of AsIRF3 and AsIRF7 were cloned into pcDNA3.1 eukaryotic expression vector according to the manufactures instruction. One microgram of pcDNA-AsIRF3 or pcDNA-AsIRF7 were co-transfected with 500 ng of Renilla luciferase reporter (phRL-CMV (Promega) and 125 ng of ISRE-luciferase (Luc) reporter construct - pISRE-Luc (Stratagene) or bi-directional NF-κB-responsive reporter construct – pSGNluc [20] into SISS cells (seabass spleen cell line, procured from National repository for fish cell lines, NBFGR, India) by Turbofect reagent. Forty eight hours after transfection, the cells were lysed using passive lysis buffer, and the firefly luciferase activity of the ISRE-Luc/pSGNluc reporter was measured and normalized by Renilla luciferase activity according to the Dual-Luciferase reporter assay system (Promega) protocol recommended by the manufacturer. Triplicate transfection experiments were repeated at least three times in order to verify the reproducibility of the results. A manual Lumat LB 9501/16 luminometer (Berthold, Germany) was used for the detection of luciferase activity.

2.8. Statistical analysis

All data were expressed as means ± SE from at least three independent experiments. The data were subjected to analysis of one way ANOVA and multiple Student t tests by using SPSS 22.0 and the P values smaller than 0.05 were considered statistically significant.

3. Results

3.1. cDNA cloning and characterization of AsIRF3 and AsIRF7

The full length cDNA sequence of Asian seabass IRF3 was found to

Table 1
Primers used in the study.

Primers	Sequence (5′-3′)
Gene cloning	
IRF3F	TCCGCTTAGTCTACAGCCCT
IRF3R	ACACTGTTACCTGATCTGCCC
IRF7F	CAGCTCAGCAGCCTTACCAC
IRF7R	TGACATTGGCGCTGTGAAGAG
RACE PCR	
IRF3-5′-R1	CGGATGGCTTCCTGTAGGTTCAC
IRF3-5′-NR1	ACCTCTCACGATGATGCTCAAA
IRF3-3′-F1	GAAAAGGACTGTGGGCAGAAATGAGG
IRF3-3′-NF1	GAGTGGCTCCCTGCCTCCATGTGAAAA
IRF7-5′-R1	GCCTTGTCATTGGGGAACTC
IRF7-5′-NR1	CACTTGCCACTGCCCATGAC
IRF7-3′-F1	ATATTCCGGTCATGGGCAGTG
IRF7-3′-NF1	ATATTCCGGTCATGGGCAGTG
UPM-long	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGTCTAAT
UPM-short	ACGACTCACTATAGGGC
NUP	AAGCAGTGGTATCAACGCAGAGT
Real-time PCR	
qIRF3-F	TCACCTGTACCGGCTGAAAC
qIRF3-R	GAACTCTGTTCGTTCCGGGT
qIRF7-F	CTTCGTCTCTTCACAGCGCC
qIRF7-R	CCATTAGAGGCACGCGAGA
IFN-1F	GCCACAAGACTAACAAGAAGC
IFN-1R	GTGAGTAGAGAAGAAACCAGC
Mx-F	GACATAGCAACCACAGAGGC
Mx-R	TCAGCCAGTTTAGGAACGGT
ISG15F	AACTCGGTGACGATGCAGC
ISG15R	TGGGCACGTTGAAGTACTGA
PKR-F	TCTTATGAGGGGACGCCAGT
PKR-R	TTAACCTGAGCCTTGGGAGG
TNF-αR	TGCTATGTAAAGCCGAGGGC
TNF-αF	GGCGCCGTACCAGTATTTCT
IL-1F	CTCATGTCTGTCCGCTACCC
IL-1R	TGAAGCTTCTGTAGCGTGGG
IL-6F	AGGAAGTCTGGCTGTCAGGA
IL-6R	CAGTGTTAACGCCACCCAGT
EF-1αF	GTTGCCTTTGTCCCCATCTC
EF-1αR	CTTCCAGCAGTGTGGTTCCA

be 2965 nucleotides consisting of 1395 bp ORF encoding proteins of 465 amino acids (Fig. 1A). The IRF3 was characterized with a 5'UTR of 250 bp, 3' UTR of 1314 bp having a polyadenylation site. Motif analysis revealed a conserved DBD which has a characteristic motif containing tryptophan pentad at its N terminus, an IRF association domain and a prominent serine rich region in the C terminus (Fig. 2A). The IRF7 transcript was found to be 2343 nucleotides containing an ORF of 1635 bp encoding 411 amino acids. ORFs are flanked by 504 bp 5′UTR and 602 bp of 3′UTR. The transcript had a polyadenylation sequence in the 3′UTR (Fig. 1B). AsIRF7 had a conserved IRF domain in the N-terminus and an IRF3 domain (IRF association domains (IAD)) (the heterodimer-binding domain of IRF3) in the C-terminal. The tryptophan cluster in the DBD comprised only four residues as homologues with other teleost IRF7 and consisted of a serine rich domain in the carboxy terminal (Fig. 2B).

3.2. Sequence comparison and phylogenetic analysis

Multiple alignments and amino acid identity comparison showed that the deduced amino acid sequences of AsIRF3 and AsIRF7 shared a high identity with homologues from other teleost species in a range of 92 to 65% overall sequence similarity. A phylogenetic tree was constructed with IRF subfamily members of various species using the neighbor joining method. The results demonstrated that AsIRF3 and AsIRF7 belonged to the IRF3 and IRF7 from other teleosts respectively with high bootstrap support (Fig. 3).

3.3. Basal expression of IRF3 and IRF7 mRNAs in tissues and developmental stages

Constitutive basal expression of AsIRF3 and AsIRF7 transcripts were observed in all the tested tissues (Fig. 4A). AsIRF3 showed its peak expression in the spleen and liver following a weak expression in muscle. The AsIRF7 expression was predominant in the gill followed by hindgut and liver with a weak expression in the muscle. The expression of AsIRF3 mRNA was detected as early as in unfertilized eggs (Fig. 4B). The expression level showed gradual increase in post-fertilized embryonic developmental stages and the maximum level was detected in neurula (9 hps) stage. The level of expression was found to be reduced in succeeding stages and the minimum level was observed following hatching (1 dph). AsIRF7 mRNA expression was detected in all the embryonic and larval stages of Asian seabass. The lowest expression of AsIRF7 mRNA was observed in 3 h post fertilization and the highest transcript abundance in neurula stage. The expression level was found to be almost similar in all the other developmental stages tested.

3.4. The expression of AsIRF3 and AsIRF7 in seabass following NNV infection in vivo

The expression profiles of AsIRF3 and AsIRF7 following NNV infection is demonstrated in Fig. 5. The pronounced fold change in the transcript of IRF3 was observed in the spleen, brain, kidney, heart and liver followed by gill in the initial stages. Brain and heart had shown significant up-regulation of IRF3 mRNA after 24 hpi and the expression were recovered to a control level at 48 hpi, however the expression of IRF3 in the kidney and liver was observed to be the highest at 72 hpi.

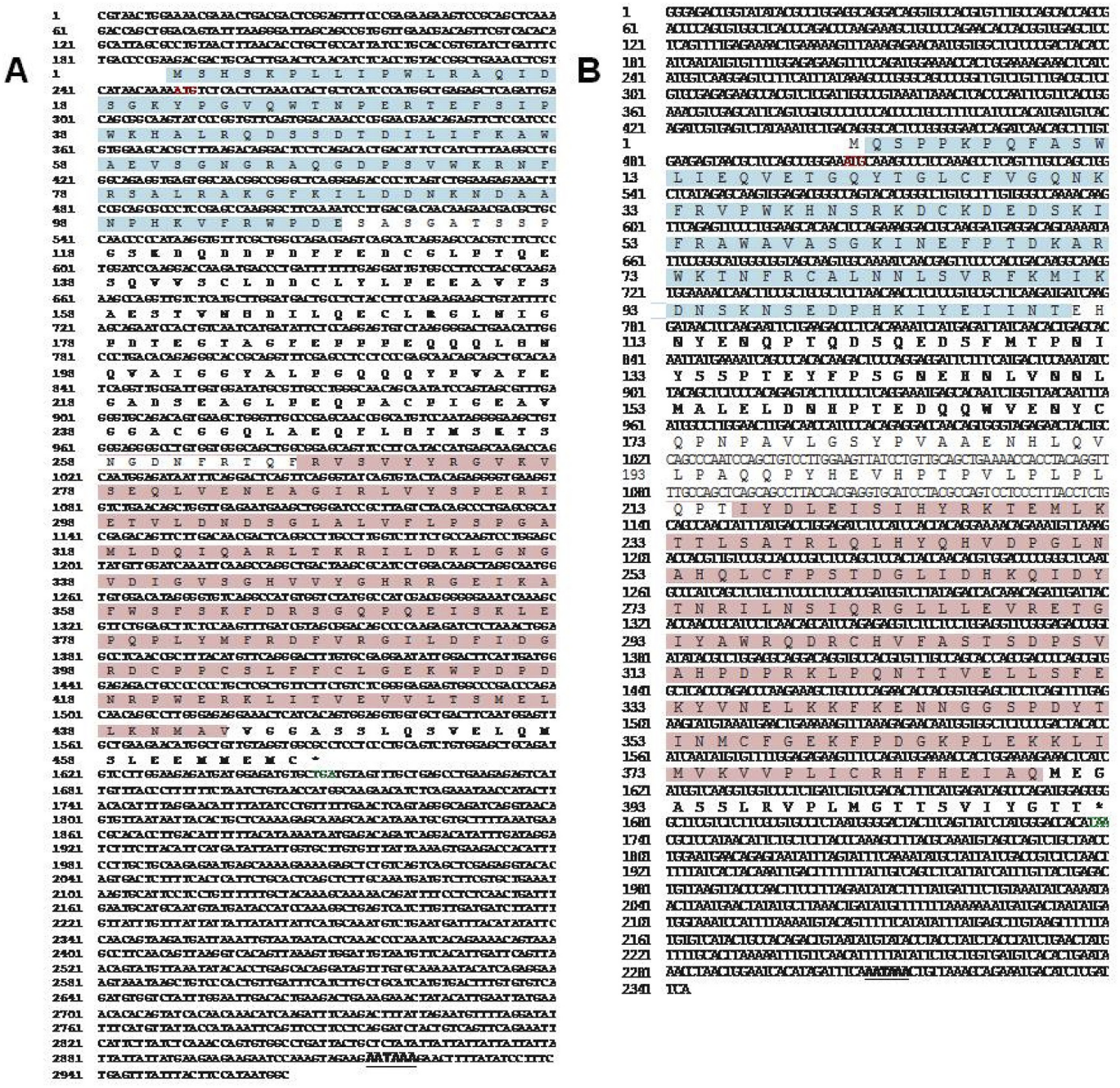

Fig. 1. The complete nucleotide and deduced amino acid sequence of Asian seabass IRF3 (A) and IRF7 (B). Blue highlight depicts IRF domain, red- IRF3 domain (IAD), bold underlined indicates polyadenylation site. Red letters is the start codon and green letters shows stop codon. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The IRF7 expression in the gill and liver was more prominent in the initial stages post challenge. As the infection progressed, the mRNA transcript abundance of IRF7 was peaked in the brain and kidney at 24 hpi and no significant change in the expression was noticed for the gill and liver. The expression recovered to control level in all the tissues at 72 hpi.

3.5. AsIRF3 and AsIRF7 induces the expression of IFN, ISGs and pro-inflammatory cytokines

To investigate the role of IRF3 and IRF7 in the regulation of IFN signaling, the SISS cells were co-transfected with ISRE promoter-driven luciferease vector and AsIRF3/AsIRF7 vector or empty vector pcDNA as control. As depicted in the Fig. 6A, transient expression of IRF3 and IRF7 resulted in an active expression of ISRE-luc up to 6.0 and 6.8 fold respectively with respective to the control. Further the over expression of AsIRF3 and AsIRF7 resulted in the increase transcript abundance of the interferon and the IFN stimulatory genes such as Mx, PKR, and ISG15 with respect to the mock and empty vector controls (Fig. 6B). The relative luciferase activity in cells co-transfected with bi-directional NF-κB-responsive reporter– pSGNluc and IRF3/7 resulted in a 4.5 and 5.0 fold induction of NF-κB promoter by IRF3 and IRF7 respectively (Fig. 6C). Moreover the results show the induction of pro-inflammatory cytokine gene transcripts in SISS cells up on ectopic expression of AsIRF3 and AsIRF7 (Fig. 6D). The expression of TNFα, IL1 and IL6 was up regulated significantly in comparison with the non-transfected and

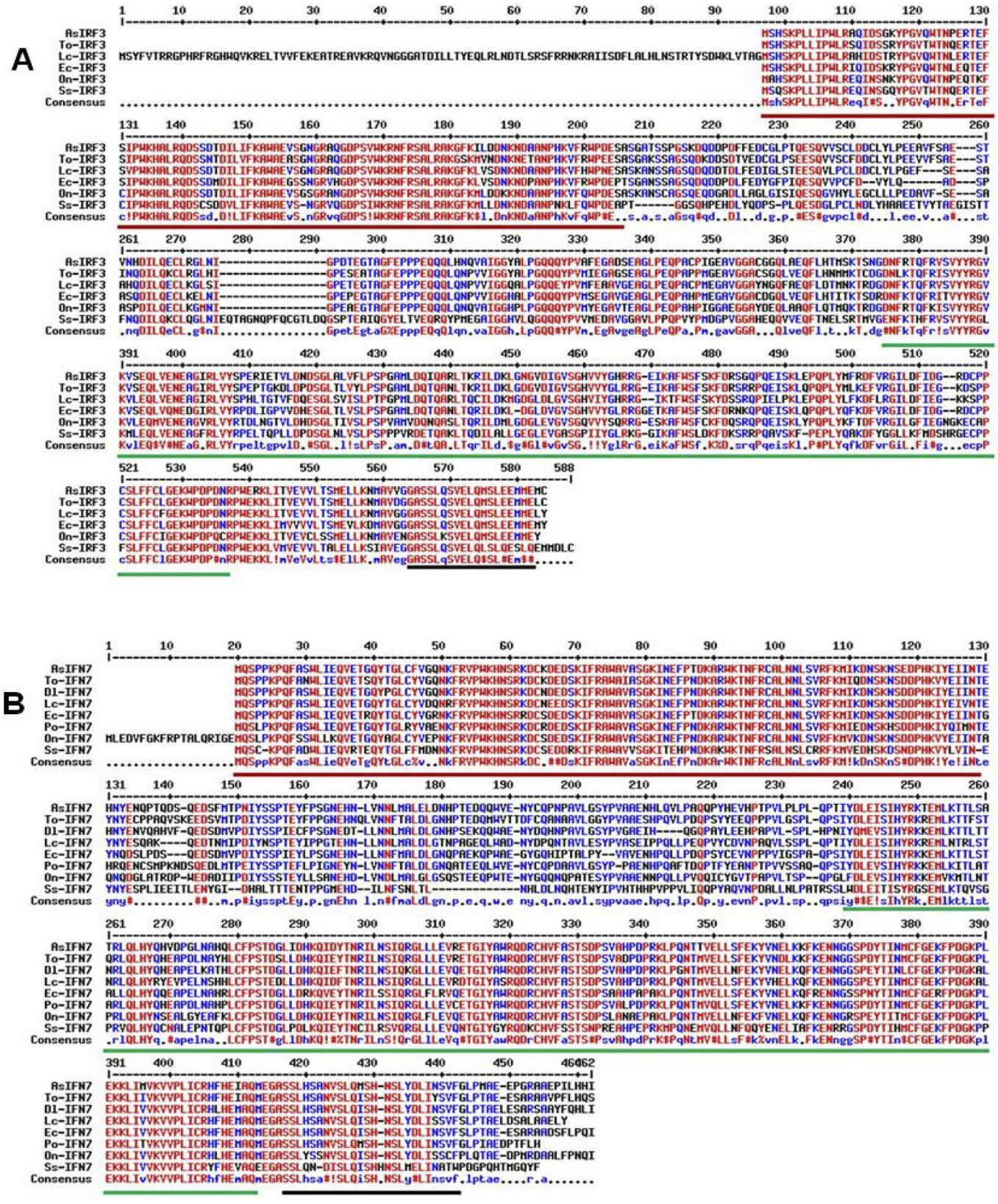

Fig. 2. Multiple sequence alignment of amino acid sequence of Asian seabass IRF3 (A) and IRF7 (B). Red underline indicates the DBD domain with 5 and 4 tryptophan residues in IRF3 and IRF7 respectively. Green shows IRF association domain and black underline shows serine rich region. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

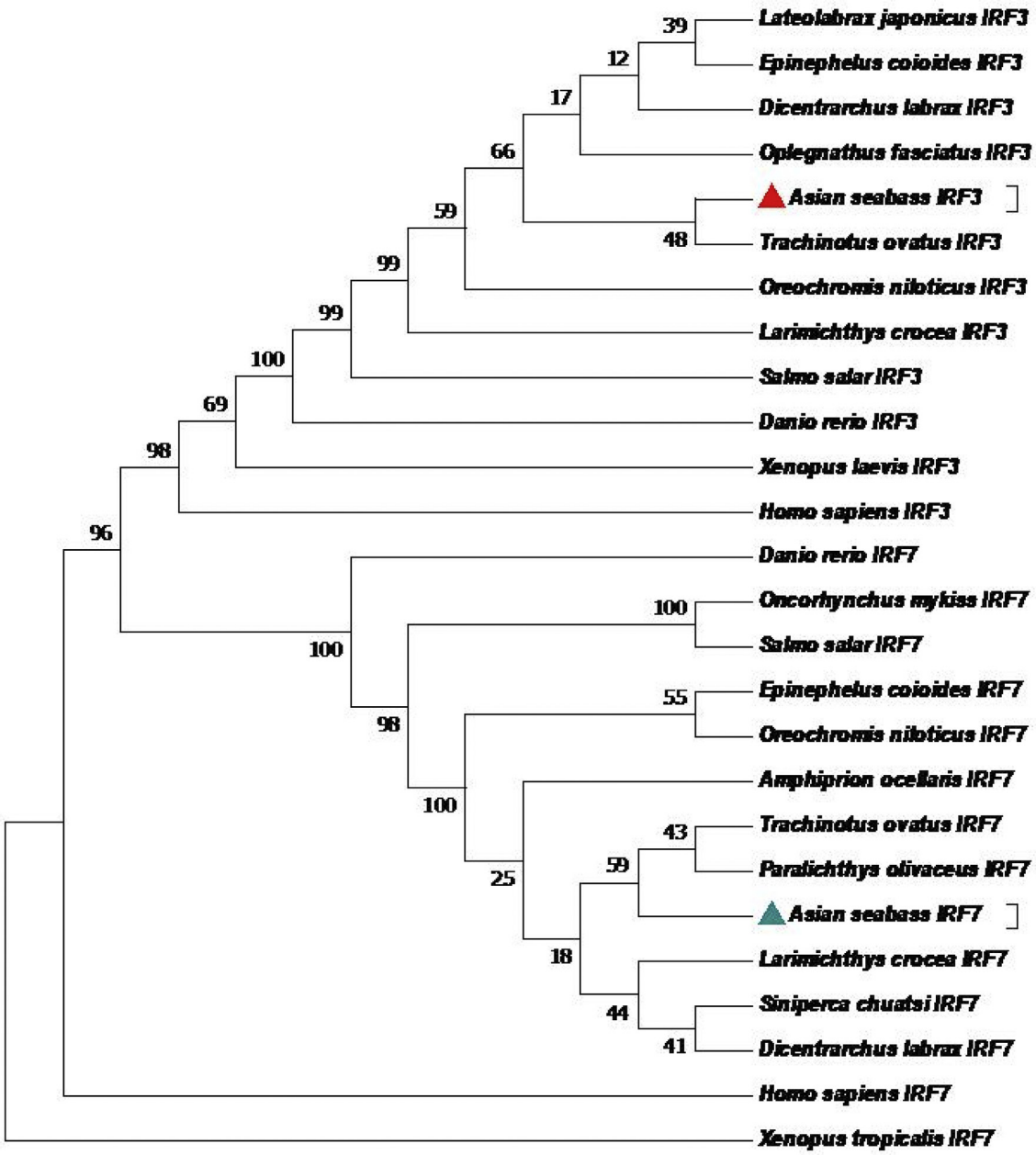

Fig. 3. Phylogenetic analysis of deduced amino acids of AsIRF3 and AsIRF7 with the other reported IRF sequences available in the GenBank. The rooted tree was constructed by the neighbor-joining method and was bootstrapped 1000 times using MEGA 7.0 software. Numbers at the nodes indicate bootstrap values.

empty vector transfected controls.

4. Discussion

The present study reports the cloning and characterization of two IRF genes from Asian seabass named AsIRF3 and AsIRF7. The IRFs are key transcription factors which are best known for its important role in the induction of type I interferon for the innate antiviral immunity [21]. Sequence analysis revealed that the characterized IRFs were true orthologes of mammalian IRFs with an N terminal DBD, C-terminal IAD and SRD. The DBD is typical of all IRF members, characterized by a cluster of five well-spaced tryptophan residues. This region forms a helix-turn-helix motif that binds to the interferon stimulating response element (ISRE) and IRF regulatory element (IRF-E) consensuses in target promoters [22,23]. AsIRF3 found to process a tryptophan pentad repeat where as the AsIRF7 had only four residues similar with other teleostean IRF7 [24–26]. The IAD is processed by IRF family members except IRF1 and 2 and it is responsible for the formation of IRF homo/hetero-dimers and associations with other transcription factors [8]. The serine rich domains was found to be conserved along vertebrates and are important for virus induced phosporylation [27]. The Ser452/Ser458 of AsIRF3 and Ser394/Ser395 of AsIRF7 were found to be putative phosporylation sites. This was concurrent with the serine/threonine cluster between amino acids 396 and 405 of human IRF-3 and Ser477 and Ser479 of human IRF-7 serine rich domains [28,29]. Considering these motif and predicted characteristic AsIRF3 and AsIRF7 have a similar activation cascade and functions as other vertebrate IRFs in regulating antiviral immunity.

Both AsIRF3 and AsIRF7 constitutively expressed in all the tissues of seabass. Similarly ubiquitous expression of IRF3/7 was observed in large yellow croaker, European eel [25,26]. The AsIRF3 transcripts were predominantly expressed in the spleen followed by liver whereas

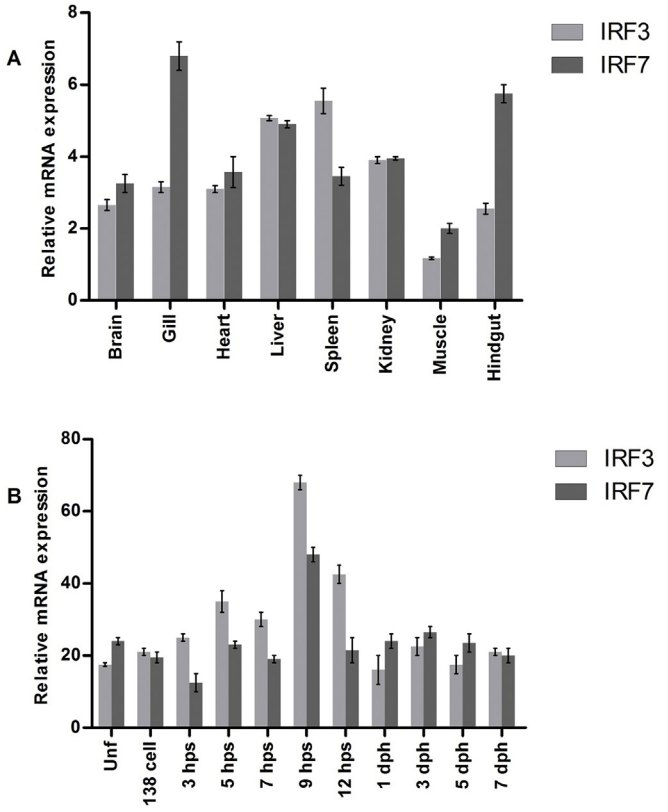

Fig. 4. Basal expression of AsIRF3 and AsIRF7 mRNA transcripts in various tissues of healthy fish (A) and developmental stages (B) of Asian seabass. The EF-1α was used as internal control. Bars represents the mean ± SEM (n = 3).

AsIRF7 expressed in the gill and hind gut similar with other teleost [25,26]. In mammals, IRF-7 is usually expressed in immune tissues; especially in plasmacytoid dendritic cells (pDCs), whereas IRF-3 is constitutively expressed in most tissue and cell types [29]. The results were in agreement with the studies on other teleosts that, all the expressed tissues were immunologically important or served as portal of pathogen entry. Fish larvae need to have defensive factors that play anti-infectious roles if they are to survive pathogen attacks before their own defense mechanisms are fully developed. The innate immune defensive factors that play protective response in early stages of fish life cycle are important in larval survival. Such factors are proteins and genes involved in the innate immune responses of fish like cytokines, transcription factors and antimicrobial peptides, which are maternally transferred to offspring through the process of vitellogenesis and oogenesis in teleosts [30,31]. The studies on ontogenetic developmental patterns of immune-related genes and transcriptional factors were undertaken in different species [32–37]. The ontogeny of IRF3/7 genes has not been studies in any species, however scarce studies on the ontogeny of IFN pathway genes are available [35,38–40]. In the current study, the expression profiles of IRF3/7 genes were evaluated in different embryonic and larval development stages of Asian seabass. The AsIRF3 and AsIRF7 were ubiquitously expressed in all stages with the highest expression noticed in the neurula stage. The transcript abundance of the two genes in the unfertilized stage suggested its maternal transfer. The results of the study were in concurrence with the previous studies on the ontogeny of RLR pathway genes of Asian seabass showing a constitutive expression in all developmental stages and high abundance of MDA5 and LGP2 in neurula stage [38–40]. These findings give a clear indication of the presence and maternal transfer of antiviral functional molecules in teleosts.

In teleosts, IRF3 and IRF7 have been identified as the key regulators of type I IFN gene expression in response to viral infection [26,41–45]. To evaluate the role of IRF3/7 in the IFN signaling pathway of Asian seabass, a challenge study with NNV was carried out. In the present study, expression level of AsIRF3/AsIRF7 was significantly up-regulated post NNV infection compared to control in all the examined tissues. The fold change of AsIRF3 expression was higher in spleen and brain in the initial stages and up regulated in heart and liver as the infection progressed. Whereas, IRF7 expression in the brain and gill was more prominent as the infection progressed. Since NNV is a neuropathogenic virus, the higher transcript expression of IRF3/7 following infection suggests the possible activation of IFN pathway in the brain tissues; however more studies needs to be performed to confirm it. However, studies in flounder have shown that IRF3 expression up on LCDV

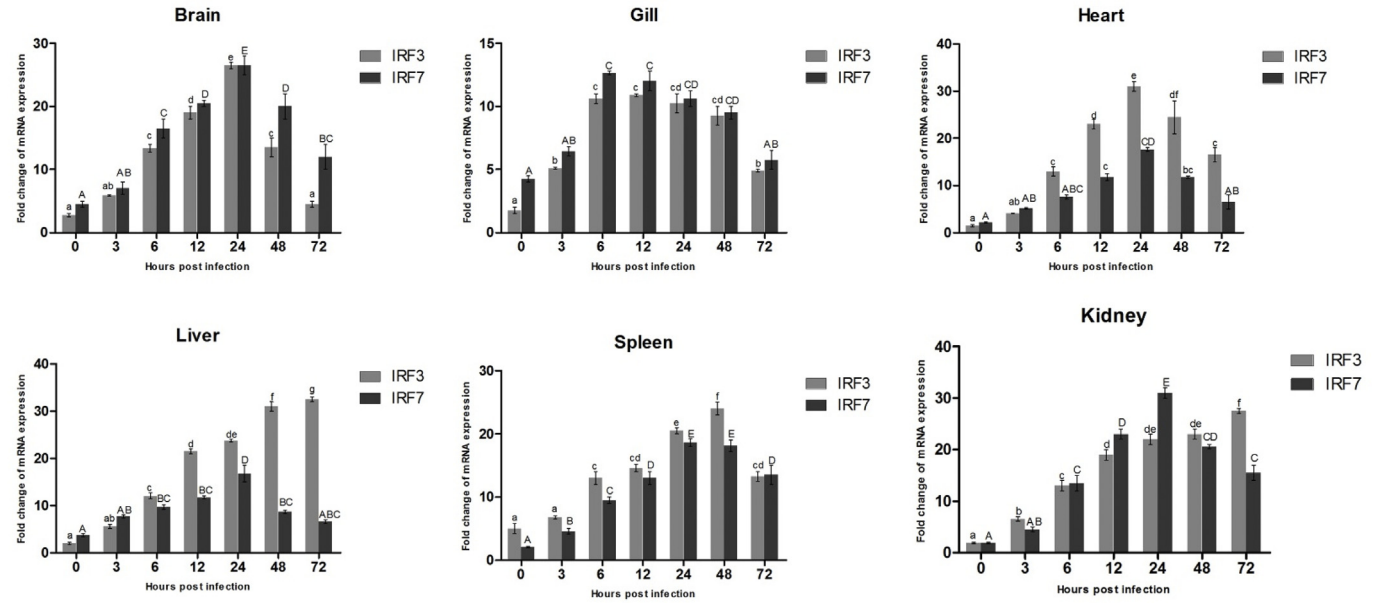

Fig. 5. Modulation of expression of AsIRF3 and AsIRF7 mRNA transcripts in different tissues at different time points after in vivo challenge with NNV, determined by qRT-PCR. mRNA levels were normalized with EF-1α mRNA levels. Bars represents the mean ± SEM (n = 3). Significant differences between the infected and the control at each sampling point were indicated with alphabets. $p < 0.05$.

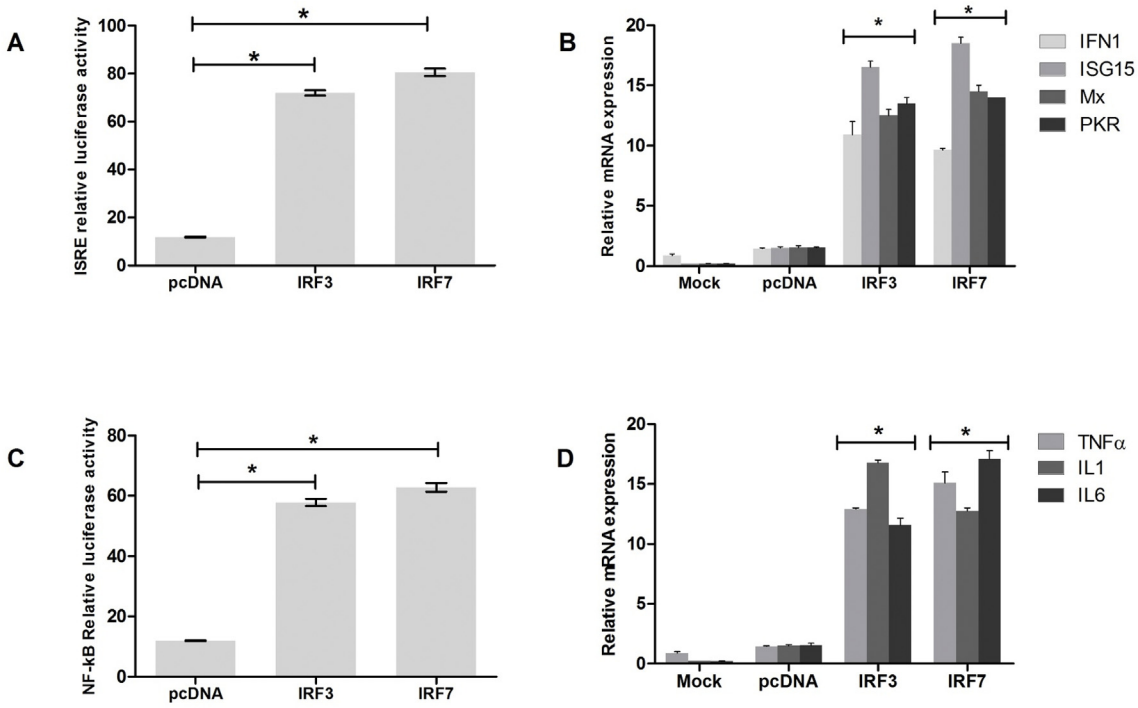

Fig. 6. Modulation of ISRE and NF-kB activity in SISS cells upon transient transfection of AsIRF3 and AsIRF7. Luciferase activity fold induction in SISS cell lines transfected with the ISRE-luc reporter plasmid (A), expression of IFN and ISGs in SISS cells transfected with IRF3/7 plasmids (B). NF-kB relative luciferase activity in IRF3/7 over-expressed cells transfected with bi-directional NF-κB-responsive reporter construct – pSGNluc (C), Transcript abundance of pro inflammatory cytokine genes in SISS cells transfected with AsIRF3/7 expression constructs. Data represented as mean ± SE of three independent experiments, Asterix (*) denotes significant difference at $p < 0.05$.

exhibited a weak induction, whereas over expression of *Epinephelus coioides* IRF3 inhibited the replication of SGIV and RGNNV [44,45]. IRF7 expression in ISAV infected salmon cells displayed heightened fold change at 72 h post infection [41], but in Asian seabass, high transcript abundance was observed at 24 h post infection in brain, heart, kidney, spleen and 6 hpi in gill. These results suggest that IRF3 and IRF7 exert an important role in the antiviral immune response in Asian seabass.

Vertebrate IRF3 and IRF7 serves as the key transcriptional factor in the downstream signaling of type I IFN-dependent immune responses in defending DNA or RNA viral infection [46–48]. Previous studies have shown that mammalian IRF3/7 activates the mRNA transcription of IFN and ISGs by binding to the ISRE on their promoters [49]. In the present study, ectopic expression of Asian seabass IRF3 and 7 resulted in the activation of ISRE promoter demonstrated with the luciferase activity. Also the expression resulted in the activation of IFN and ISGs. Similar results were obtained in a study of rainbow trout IRF3/7 showing increased luciferase activity of Mx promoters [14]. Induction of IFN promoters up on IRF expression was also demonstrated in other species such as flounder, Atlantic salmon and tilapia [41–43,50]. There were reports suggesting the interactions of IRFs and NF-κB promoters such that, the IRF3 mediated activation of ISRE in TLR3 signaling required the p65 subunit of NF-κ [51]. In our study, we observed an interaction between AsIRF3/AsIRF7 with NF-κB promoter demonstrated by an induced luciferase activity and expression of pro-inflammatory cytokine genes. However further studies are warranted to understand the actual interaction between the IRFs and NF-κB in the activation of pro-inflammatory cytokines. These findings suggest that AsIRF3 and AsIRF7 are the major transcription factors involved in the IFN mediated antiviral defense in Asian seabass.

In summary the full length cDNA sequence of IRF3 and IRF7 of Asian seabass was cloned and characterized. The deduced amino acid sequence displayed homology with their other vertebrate counterparts with similar domain organization. The AsIRF3 and AsIRF7 were found to transfer maternally and expressed ubiquitously in all the tissues of fish. Induced expression f the both genes were observed in the NNV challenged fish with high abundance in the bran post challenge. Further the function analysis revealed that the IRF3/7 induced the expression of IFN, ISGs and pro-inflammatory cytokines and play an important role in the interferon mediated antiviral immune response in the Asian seabass.

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