ABSTRACTS

SESSION 7: GENETICS OF DISEASE AND STRESS

COULD GENOMIC EVALUATIONS PREDICT RESISTANCE TO VIRAL NERVOUS NECROSIS IN WILD POPULATIONS OF EUROPEAN SEABASS ?

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Abstract

Viral Nervous Necrosis (VNN) is a major threat for European sea bass aquaculture in the Mediterranean Sea (Doan et al. 2017a). While no efficient vaccination or possible prevention method allow to prevent the disease, we explore genomic routes to obtain more resistant populations: marker-assisted or genomic selection. However, these breeding technics require still some research to be applied for VNN resistance in European sea bass in particular by either highlighting relevant genes to target or evaluating the interest of genomic-based estimation of breeding values. After the experimental infection of 1472 offspring derived from a full factorial mating of nine dams cross with 60 sires from four different wild populations of European sea bass, we demonstrated large differences in the survival of sea bass exposed to VNN, from 44% to 99% (Doan et al 2017b). Parents and offspring were genotyped on a custom Illumina Iselect® 3K SNP array. 1274 informative SNPs were retained to detect QTLs of NNV resistance by a weighted (wGWAS) with BLUPF90, based on a single trait linear mixed model. The wGWAS highlighted 3 putative QTLs explaining a significant percentage of variance of the NNV resistance: LG9 (3.7%), LG12 (6.2%) and LGx (3.4%). Genomic models for estimating breeding values (GBLUP and SNP-BLUP) were fitted in BLUPF90 and GS3 to evaluate the accuracy of genomic prediction, compared to pedigree-based (PBLUP) method. The accuracy of breeding values predicted with genomic models (R_{GBLUP} = 0.76 and $R_{SNP\text{-}BLUP}$ = 0.74) were no different from those of PBLUP (R_{BLUP} = 0.78). However, the genomic heritability (0.14±0.05) was estimated as half of the polygenic heritability (0.23±0.08). This suggests that our experimental design was suboptimal to account for NNV resistance variation using genomic information. NNV resistance could be more efficiently improved with genetic evaluations using high densisty SNP genotyping (i.e. with the further commercialized 57K AxiomDlabCHIP) in more suitable mating designs using larger families within one population.

Keywords: VNN, Sea bass, wGWAS, GBLUP

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RESPONSE OF THE SALMON TRANSCRIPTOME TO PANCREATIC DISEASE: COMPARISON OF HIGH- AND LOW-RANKING FAMILIES FOR RESISTANCE

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Abstract

Outbreaks of pancreas disease (PD) caused by salmonid alphavirus (SAV) affect salmon and trout in Norway, Scotland, Ireland and North America. Outbreaks lasting 3-4 months regularly occur in Norway, but as there are no obvious external signs of initial infection, control is difficult. Heritability of resistance to PD is high (~0.4 in post-smolts, ~0.5 in fry) [1] and QTL affecting resistance have been detected [2]. The aim of this study was to compare the transcriptome of animals with high and low resistance to PD, based on estimated breeding values (EBV), before and after experimental challenge with SAV, to detect genes and pathways that are differentially expressed and to gain knowledge about the biological basis for resistance to the disease. Naïve fish at parr stage (average weight 37g) with either high or low EBV for PD resistance (PDR) were selected based on family EBV's (from 10 families and 4 groups, half with high and half with low PDR-EBV). The fish were acclimatised in fresh water tanks at VESO Vikan Norway before challenge with SAV3. Half of the fish were challenged with intraperitoneal (IP) injection. The other half were infected one week later by using the IP challenged fish as shedders under a cohabitation challenge test model. Heart was sampled for gene expression (mRNA-seq) and later DNA-methylation (RRBS) analysis (15 high and 15 low PDR-EBV naïve fish at 0 weeks and 5 fish per EBV ranking and challenge model at 4 and 10 weeks post infection). Large numbers of genes were significantly differentially expressed between high and low PD resistance EBV ranked fish 4 and 10 weeks after CH challenge (1804 and 949 for the cohabitant and IP challenges respectively 4 weeks post-infection). Six genes (of which 5 were annotated as class I histocompatibility antigen, actin 2C alpha cardiac muscle 1, immunoglobulin kappa-b4 chain C and disks large homolog 2) were differentially expressed in naïve fish and across the time course of both challenge tests. Analysis of gene ontology enrichment revealed that differentially expressed genes were mainly involved in: cardiovascular related biological processes in naïve fish; translational processes (initiation, elongation and termination) 4 weeks after co-habitant challenge; immune system processes 10 weeks after co-habitant challenge; circulatory system, glucose metabolism, blood vessel morphogenesis and cardiovascular development 4 weeks after IP injection and; ageing, negative regulation of cell death, response to inactivity and cardiovascular biological processes 10 weeks after IP injection. Results will be discussed in the context of findings by other authors [3, 4] showing that the immune system responds faster when animals are injected, and that the initial innate immune response is critical for controlling the acute phase of SAV infection. This is part of a broader study with the aim of further developing and applying genomic tools for breeding to increase resistance to PD in Atlantic salmon.

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Keywords: Pancreas disease, resistance, differential gene expression, Atlantic salmon, mRNA-seq

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IDENTIFICATION AND EVALUATION OF LONG NON-CODING RNAS RESPONSE TO HANDLING STRESS IN RED CUSK-EEL (*GENYPTERUS CHILENSIS*) THROUGH RNA-SEQ.

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Abstract

The red cusk-eel (*Genypterus chilensis*) is a native species with high potential to Chilean aquaculture diversification. In commercial conditions, fish are submitted to several stressors, affecting the performance of fish. However, the knowledge related to mechanism involved in stress response in *G.chilensis* is scarce, with no information related to the regulation mediated by long non-coding RNAs (lncRNA). The objective of this work was: (i) identify for first time lncRNA in the transcriptome of *G.chilensis*; (ii) evaluate the correlation in the expression between lncRNA and coding-RNA in liver, head-kidney and muscle of two groups of *G.chilensis* (control and handling stress groups); and (iii) determine the differential expression of lncRNA in these three tissues in response to handling stress. We use a previous published reference transcriptome to identify lncRNAs, using a series of successive filters based in several databases annotation to discard coding sequences. We identify a total of 14737 putative lncRNA in the transcriptome of *G.chilensis*, providing a useful lncRNA reference resource to be used in future studies. We mapped the read of each library to the reference transcriptome with CLC platform, obtaining the expression values. We evaluate the correlation between lncRNA and coding-RNA considering the expression across all tissues in control and stress groups, identifying the lncRNA differentially expressed through a proportion test (Kal's Z-test). We identify 125, 405 and 128 differentially expressed lncRNA between control and stress group in liver, head-kidney and muscle, respectively. An enrichment analysis of coding-RNAs correlated with the lncRNAs showed an over-representation of lncRNA-correlated genes with oxidation-reduction process and lipid metabolic process in liver, with no enriched terms found in muscle or head-kidney. In muscle, we found lncRNA-correlated genes involved in ubiquitin-mediated proteolysis, proteasome, muscle contraction and growth. In head-kidney, we found lncRNA-correlated genes involved in cytokine-cytokine interaction, RAS, MAPK, mTOR and TNF signaling. This study showed that lncRNA are modulated by handling stress in all tissues evaluated, evidencing how the stress affect several metabolic processes in coding and non-coding RNA level, suggesting a role of lncRNA in stress response that should be deeply studied. Funding: CONICYT FONDECYT Postdoctorado 3180283, CONICYT FONDAP 15110027.

Keywords: long non-coding RNA, Genypterus chilensis, red cusk-eel, RNA-seq, stress response

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HERITABILITY ESTIMATES AND SELECTION RESPONSE FOR RESISTANCE TO *STREPTOCOCCUS AGALACTIAE* IN RED TILAPIA

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Abstract

Streptococcosis is considered the most devastating disease in tilapia farming worldwide. The causative agent has been identified as *Streptococcus agalactiae* (SA), a Gram-positive bacterium. This study presents results of selection response to SA resistance in a commercial stock of red tilapia (*Oreochromis* spp.) after one generation of selection. Families were produced from 93 males and 128 females using a nested design (1 male: 2 females). At 45 days post-hatch, fry were marked with PIT tags and reared until 60 days post-hatch. Thirty fish from each family were challenged by intraperitoneal injection of bacterial solution at the 96 h LD_{50} concentration (1×10^9 CFU/mL) and observed for 14 days. Disease resistance was measured as a binary trait (dead/alive) and as a continuous trait, i.e., survival time, or the number of days from challenge initiation until death (DD). Data from 128 full-sib and 35 half-sib families was used to estimate variance components using ASREML version 4.0. Animal, sire and sire-dam models were used for DD, while sire and sire-dam models were used for binary outcomes. Survival rates in G0 ranged from 0 to 60% with a mean of 16.52 ± 1.02%. Only two families exhibited survival rates over 50%. Breeding candidates in the base population (G0) were selected from the top 10 families and used to produce 25 full- and 6 half-sib families. Heritability estimates for G0 were highest under the sire model, 0.31 ± 0.12 for binary data and 0.28 ± 0.08 for DD. Similar estimates (0.20 ± 0.03) were obtained from animal and sire-dam models for DD. The sire model for DD performed the best with r_{EBV} = 0.663 and accuracy of selection = 0.81. When data from G0 and G1 were combined, heritability estimates were 0.29 ± 0.11 and 0.27 ± 0.08 for binary and DD outcomes for the sire model. The selection response was 19.77% for survival time. The results indicate there is significant selection potential for increased disease resistance to *S. agalactiae* in red tilapia.

Keywords: *streptococcosis, binary trait, sire model, sire-dam model, predictability*
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MARKER-ASSISTED SELECTION FOR RESISTANCE TO BACTERIAL COLD WATER DISEASE IN A COMMERCIAL RAINBOW TROUT POPULATION

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Abstract

Bacterial cold water disease (BCWD), caused by *Flavobacterium psychrophilum*, is an endemic and problematic disease in rainbow trout (*Oncorhynchus mykiss*) aquaculture. Previously, we have identified SNPs (single nucleotide polymorphisms) associated with BCWD resistance in rainbow trout. The objectives of this study were 1) to validate the SNPs associated with BCWD resistance in a commercial breeding population; and 2) to evaluate retrospectively the accuracy of MAS (marker-assisted selection) for BCWD resistance in this commercial breeding program. The Troutlodge May breeding population was evaluated for BCWD resistance in three consecutive generations, 2013TLUM, 2015TLUM and 2017TLUM. Based on our previous studies, a panel of 96 SNPs was selected and used to genotype the parents and ten offspring from each of the 138 full-sib families of the 2015TLUM generation, and 37 SNPs associated with BCWD resistance were validated. Thirty-six of the validated SNPs were clustered on chromosomes Omy3, Omy8 and Omy25. Thus, at least three QTL (quantitative trait loci) for BCWD resistance were validated in the 2015TLUM generation. Three SNPs from each QTL region were used for haplotype association analysis. Three haplotypes, Omy3TGG, Omy8GCG and Omy25CGG, were found to be associated with BCWD resistance in the 2015TLUM generation. Retrospective analyses were then performed to evaluate the accuracy of MAS for BCWD resistance using these three favorable haplotypes. The accuracy of MAS was estimated with the Pearson correlation coefficient between the total number of favorable haplotypes in the two parents and the family BCWD survival rates. The Omy8 and Omy25 haplotypes were positively correlated with the family BCWD survival rates across all three generations. The accuracies of MAS using these two haplotypes together were consistently around 0.5, which was equal or greater than the accuracy of the conventional family-based selection in the same generation. In conclusion, we have demonstrated that MAS for BCWD resistance is feasible in this commercial rainbow trout breeding population.

Keywords: rainbow trout, bacterial cold water disease, haplotype, SNP, MAS

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ATLANTIC SALMON miRNAs RESPONDING DIFFERENTLY TO *INFECTIOUS PANCREATIC NECROSIS VIRUS* (IPNV) INFECTION IN FISH SELECTED FOR RESISTANCE OR SUSCEPTIBILITY TO IPNV INFECTION

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Abstract

MicroRNAs (miRNAs) control multiple biological processes including innate immune responses by negative post-transcriptional regulation of gene expression. The objective of this study was to identify miRNAs responding to *infectious pancreatic necrosis virus* (IPNV) infection. Materials consisted of fish classified as resistant (RR) or susceptible (SS) by genotyping the major quantitative trait locus associated with resistance to IPN in Atlantic Salmon. Fish were collected at 1, 7 and 20 days post challenge (poc) to identify miRNAs responding at different time points. *In silico* prediction of target genes was carried out to identify putative targets among immune pathway genes. RNA extracted from whole fry (n=96) was submitted to Illumina small RNA sequencing. DESeq2 was used to identify differentially expressed miRNAs (DE miRNAs) and TargetScan, miRanda, PITA and RNAhybrid were used to predict target genes. Twenty-two microRNAs belonging to seventeen miRNA families were differentially expressed at one or more of the time points poc when compared to healthy controls. Four miRNAs showed early increased expression (1 day poc), while 10 miRNAs showed decreased expression at 7 days poc. At the latest timepoint, six miRNAs showed increased, while two showed decreased expression. *In silico* analysis revealed that a subset of the DE miRNAs could target important genes associated with immune response and immune signaling pathways such as IRFs, IFNs and TLRs. This indicates that they are likely to have important roles in host immune responses by regulating key genes in immune gene networks. Dividing materials into RR and SS individuals revealed eleven additional miRNAs (a total of 17) that differed at the latest timepoint. Sixteen miRNAs showed large increases, while one showed a large decrease, in susceptible *vs* resistant individuals compared to normal expression (controls). This is the first observation of a difference in expression of A. salmon miRNAs in resistant versus susceptible individuals following viral infection. Knowledge on the role of these miRNAs, and DE miRNAs identified at other time points poc, depends on further experimental identification of true target genes. The ones predicted from *in silico* analysis would be first candidates to test in such experimental assays.

Keywords: micoRNA, Atlantic salmon, innate immune response, post-transcriptional regulation
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FUNCTIONAL CHARACTERIZATION OF KAZAL TYPE PROTEASE INHIBITORS FROM SEBASTES SCHLEGELII AND HIPPOCAMPUS ABDOMINALIS: MOLECULAR INSIGHT INTO HEPATIC IMMUNE DEFENSE IN TELEOST

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Abstract

Kazal-type serine protease inhibitor (KSPI) is one of the serpins, which plays a vital role to regulate endogenous proteases, cell development, blood coagulation and immune response. In this study, we identified and characterized two different KSPI homologues from black rockfish (*SsKSPI*) and big-belly seahorse (*HaKSPI*) respectively. The full-length of *Ss*KSPI was 532 bp, including open reading frame (ORF) of 330 bp which encodes a polypeptide of 110 amino acids with a signal peptide of 21 amino acids. The greatest value of the identity (42.9%) and similarity (50.9%) was observed with *Channa striatas*. On the other hand, the full-length cDNA sequences of *HaKSPI* was 333 bp. The 255 bp of ORF encoded a protein of 85 amino acids. The Homology studies indicated that *HaKSPI* showed the highest identity (84.7%) and similarity (89.4%) with KSPI of *Hippocampus comes*. qRT-PCR results showed that *Ss*KSPI and HaKSPI ubiquitously expressed in many tissues at different levels. Remarkably, Two KSPI transcripts in liver showed the highest expression ($P < 0.05$). The biotic and abiotic challenges were conducted and the liver tissue was assessed for the gene modulation by qRT-PCR. The mRNA expression of *Ss*KPI was significantly up-regulated throughout the challenge in response to *S.iniae*, LPS and Poly I:C. The mRNA expression of *HaKSPI* in liver was induced upon *S.iniae*, *E.tarda*, LPS and Poly I:C challenges. The recombinant protein of SsKSPI and HaKSPI were produced to characterize and study the KSPI specific functions. The recombinant protein of SsKSPI and HaKSPI strongly inhibited subtilisin compared to other tested proteases. Moreover both recombinant proteins possessed the bacteriostatic activity against Gram-positive bacteria, *Streptococcus iniae* and Gram-negative bacteria, *Edwardsiella tarda*. Taken together, our results suggest that *SsKSPI* and *HaKSPI* may play an essential role in hepatic immune response.

Keywords: Kazal-type protease inhibitor, Sebastes schlegelii, Hippocampus abdominalis, immune response, mRNA expression

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RESISTANCE TO *FLAVOBACTERIUM PSYCHROPHILUM* IN RAINBOW TROUT: QTL DETECTION REVEALS EFFECT OF INFECTION ROUTE AND EPISTATIC INTERACTIONS

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Abstract

Bacterial cold-water disease, caused by *Flavobacterium psychrophilum* (Fp) is one of the most important diseases affecting rainbow trout (*Oncorhynchus mykiss*). Previous studies that used injection as route of infection have revealed that resistance to Fp is controlled by several QTL. However, skin and mucus are recognized as important components of fish defense against infections. Immersion challenge is expected to be closer to natural infection by Fp, and may reveal new defense mechanisms. The objective of this study was to identify and compare QTL associated with resistance to Fp using the two routes of infection. Three experimental families, one F2 progeny and two gynogenetic doubled haploid, were produced in RE-SIST and FISHBOOST projects (FUI 15 French and FP7 European) from 6 doubled haploid grand-parents belonging to 6 isogenic lines with opposite resistance to Fp (3 resistant, 3 susceptible). Around 300 to 600 fish/family were individually tagged and challenged using either intramuscular injection or immersion (survival rate from 7% to 50% for injection; from 30% to 80% for immersion). Fish were genotyped with 1,200 to 9,600 markers using the double-digest-RAD or RAD-sequencing methodology. QTL for different resistance traits (i.e. survival, tolerance or endurance) were detected separately for each family and each infection route, using linkage analysis and interval mapping method. Several chromosome- and genome-wide significant QTL ($P<0.01$ and $P<0.05$, respectively) associated with resistance to Fp were detected. Most of them were family specific but the most significant were detected in several families. Many QTL were detected after either immersion or injection. Only a few were found after both infectious challenges, suggesting that different defenses mechanisms may be triggered depending on the infection route. Interestingly, epistatic interactions among resistance QTL were evidenced, highlighting the complexity of the underlying immune pathway. This study confirms the complex genetic architecture of trout resistance to Fp and demonstrates that different defense mechanisms can be modulated by the infection protocol. Some QTL with large effect were identified consistently across different genetic backgrounds and infectious protocols, paving the way for a better understanding of host defense mechanisms and further implantation of selective breeding for resistance to Fp.

Keywords: BCWD, QTL, Resistance, Route of infection, Epistasis

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GENOMIC APPROACHES TO UNDERSTANDING AND IMPROVING RESISTANCE TO SALMONID RICKETTSIAL SYNDROME IN ATLANTIC SALMON

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Abstract

Salmonid Rickettsial Syndrome (SRS) is a bacterial disease which can cause high mortalities in Atlantic salmon aquaculture. The etiological agent is the intracellular bacterium *Piscirickettsia salmonis*, which infects several tissues causing inflammation, necrosis and eventually death. Understanding the host-pathogen interaction and developing SRS resistant stocks via selective breeding can lead to better control of this disease. The aims of this study were to a) evaluate the heritability of host resistance to SRS, b) study the genetic architecture of the trait, c) assess the potential of genomic selection using imputed genotypes, d) increase our understanding of the host response through RNA-Seq, and e) find candidate genes for resistance to SRS. To achieve this, an SRS challenge experiment was performed on Atlantic salmon from a Chilean commercial breeding program. ~2,500 fish were genotyped using a 1K SNP panel and imputed to high density (~40K), using the high density genotypes of ~1,000 full-siblings. In addition, liver and head kidney samples for control and SRS infected fish at 3 and 9 days post infection (~20 samples per tissue and condition) were collected for RNA sequencing. These samples were also genotyped using the 1K SNP panel. Heritability of SRS resistance was estimated at 0.43 ± 0.04. A genome-wide significant SNP was detected on chromosome 2, but the trait appears to have a largely polygenic architecture. The selection accuracy of GBLUP using imputed high-density genotypes was not better than that of GBLUP with the low density panel, however Bayesian methods resulted in ~7% accuracy increase with the imputed genotypes (Figure 1). The gene expression signature of host response to SRS was clear from a principal component analysis, with the first component explaining 24 and 57% of the variance in liver and head kidney respectively (Figure 2). Based on their EBVs for SRS resistance, samples from resistant and susceptible salmon were compared, resulting in several interesting candidate genes potentially involved in SRS resistance, including members of the complement pathway. This study improves our understanding of the host response and resistance to SRS, and highlights the potential of cost-effective genomic selection to obtain more resistant stocks.

Keyworkds: Atlantic salmon, Piscirickettsia salmonis, GWAS, genomic selection, RNA-Seq

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Figure 1. Genomic selection SRS mortality

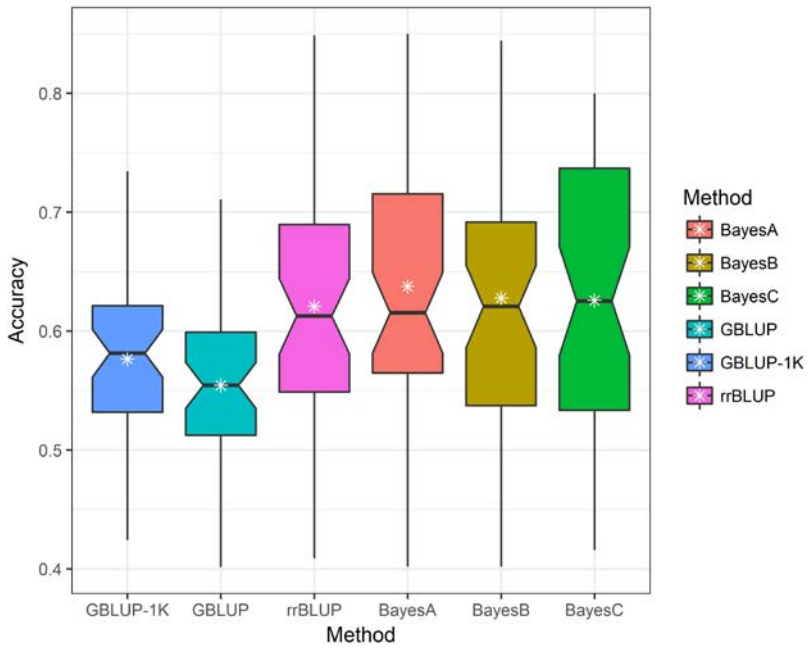
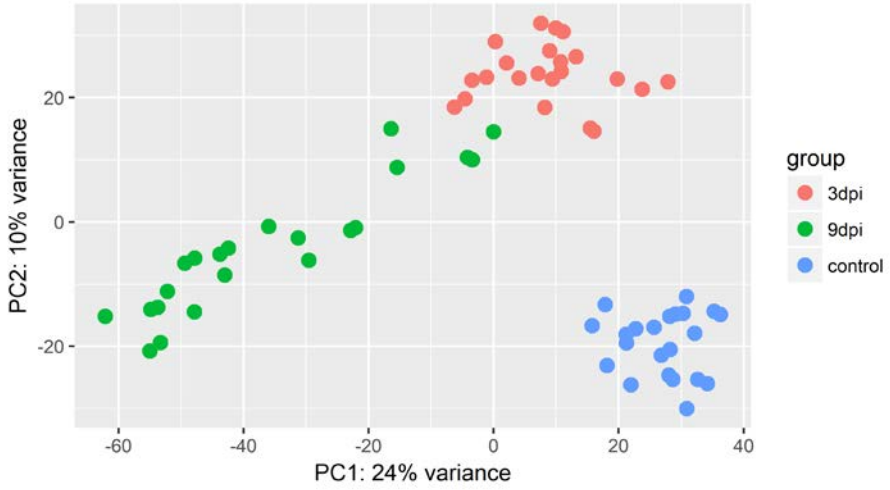


Figure 2. PCA liver RNA-Seq



HYPOXIA-TOLERANT PERFORMANCE IN SELECTED BREEDING F_4 STRAIN OF BLUNT SNOUT BREAM (*MEGALOBRAMA AMBLYCEPHALA*) UNDER HYPOXIA STRESS

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Abstract

In this study, we found that the F_4 generation after selected breeding from the Poyang lake population and 'Pujiang No.1' control by hypoxia stress have markedly hypoxia-tolerant performance. Compared to the 'Pujiang No.1' blunt snout bream control, the hypoxia-tolerant F_4 strain had a significantly ($P < 0.01$) low critical oxygen tension at which they lost their equilibrium (LOE_{crit}) at 10 °C, 25 °C and 30 °C, respectively. When the hypoxia-tolerant strain were exposed to 4- or 7-days of hypoxia at 10 °C, the average protruding lamella heights and mean lamellar area of gills were significantly ($P < 0.01$) smaller than 'Pujiang No.1' control. These changes resulted in increased average interlamellar cell mass (ILCM) height and volume in hypoxia-tolerant strain under hypoxia. These results suggested that hypoxia-tolerant strain has a greater potential for coping with further hypoxia stress. Simultaneously, in order to enhance blood oxygen-carrying capacity to adapt hypoxic environment, the blood erythrocyte count and haemoglobin (Hb) concentration of hypoxia-tolerant strain increased significantly ($P < 0.01$) than those in 'Pujiang No.1' control during hypoxic treatment. Taken together, our studies demonstrate that the selected F_4 strain has the potential for breeding a hypoxia-tolerant blunt snout bream cultivar for future use.

Keywords: Megalobrama amblycephala; Hypoxia-tolerant strain; Gill remodeling; Hemoglobin

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