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Comparison of stress tolerance of hybrid and selfed offspring of two populations of *Litopenaeus vannamei*

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In recent years, as the marine environment has been deteriorated, the aquaculture water environment has also been negatively affected to varying degrees. Negative environmental factors make extremely damaging to organisms, resulting in stress-induced diseases and high mortality rates of cultured shrimp. Therefore, stress resistance breeding of Litopenaeus vannamei and evaluating the stress tolerance of the breeding population are urgently needed now. Litopenaeus vannamei collected from Thailand (T) and the United States (M) were used as parents, while four progeny populations of TT, MM, TM, and MT were constructed by diallel cross, with a total of 20 families. The tolerance of young shrimp to high ammonia-N, high pH, and low salt was compared through a 96-h acute toxicity test; the heterosis of each mating combination was analyzed; and the tolerance of parents and offspring was evaluated. Here we show that under 96 h of high ammonia-N, high pH, and low salt stress, the mortality rates of each family were 19.52%–92.22%, 23.33%–92.22%, and 19.33%–80.00%, respectively. There were significant differences in the tolerance of different families to ammonia-N, pH, and salinity stress ($P < 0.05$). The population with a female parent from the United States had stronger tolerance to ammonia-N, pH, and low salt stress than the population with a female parent from Thailand. The population with a male parent from Thailand had weaker tolerance to pH and low salt stress than the population with a male parent from the United States, but it was superior to the population with a male parent from the United States under ammonia-N stress. The heterosis rates of the hybrid population TM in acute high ammonia-N, high pH and low salinity were 81.67%, 44.58% and –10.13%, respectively; However, the heterosis rates of the MT population were 14.89%, 38.89%, and –8.96%, respectively. The overall resistance of the four populations showed MM > MT > TT > TM. The population TM had obvious heterosis in high ammonia-N and high pH tolerance traits, and the family MM7 had a strong low salt tolerance, so it can be considered a candidate family for subsequent breeding work. Moreover, variability in stress resistance and heterosis of stress resistance of different populations obtained by family selection was discussed in this paper. The experimental results provide a basis for screening new strains of vannamei shrimp with strong stress resistance through family breeding.

KEYWORDS
Litopenaeus vannamei, ammonia-N, pH, salinity, tolerance, heterosis

Introduction

The Pacific white shrimp, *Litopenaeus vannamei*, is naturally distributed in tropical waters off the Pacific Ocean from Peru to Mexico, from the Gulf of Mexico to central Peru, especially close to Ecuador (Zhang et al., 1990). It benefits from small head and chest armor, high meat content, strong stress resistance, fast growth, a long breeding period, high density and low salinity tolerance for aquaculture, and simple transportation of live shrimp. Along with *Fenneropenaeus chinensis* and *Penaeus monodon*, it is listed as one of the world's three most widely cultivated shrimp species and is crucial to both shrimp fisheries and aquaculture (Wang et al., 2004; Zhang et al., 2009).

For the sustainable development of marine aquaculture, a healthy marine environment is a prerequisite. Important environmental factors in the shrimp farming environment include ammonia-N, pH, and salinity. However, stress-induced diseases of cultured shrimp are common due to the deterioration of the marine environment, particularly in coastal areas, the pollution of the water environment caused by high-density aquaculture, and the mutation of the aquaculture water environment caused by many external factors (especially rainstorms, typhoons, cold waves and other adverse weather), and such problems have not yet been effectively solved (Huang et al., 2012). Negative environmental factors can disrupt the natural physiological balance of organisms, and sustained environmental stress can destroy organs and tissues irreparably or even eliminate an organism (Dunier and Siwicki, 1993; Chen et al., 1995; Chen and Chen, 2003). These effects result in high mortality rates and huge economic losses. One of the best ways to deal with these problems is through genetic improvement of stress resistance indicators such as ammonia-N, pH, and salinity in *L. vannamei* (Hu et al., 2016).

One of the most important methods for genetic improvement in aquatic animals is cross-breeding (Zhang et al., 2019). Their offspring can outperform their parents in terms of production performance, environmental tolerance, and meat quality by crossing individuals of different strains or genotypes (Piferrer et al., 2009). Because they can quantify the level of heterosis obtained after crossing different lines and reflect the matching effect of target traits, heterosis and combining ability are very important references in breeding. Zhou (Zhou et al., 2021) studied the heterosis and combining ability of body mass traits of three populations of *Penaeus monodon* through a complete diallel cross design. Sun (Sun et al., 2011) discovered significant differences in high ammonia-N tolerance among various families of *Penaeus monodon*, and He (He et al., 2008) found that there were significant differences in the resistance of juvenile shrimp seedlings among Chinese shrimp families. Different families have significant differences in tolerance to ammonia-N and pH, indicating significant selection potential. Fang (Fang et al., 2000) discovered that as pH increased, fewer juvenile Chinese shrimp survived. Acute salinity stress has been shown to affect the growth and survival rate of *L. vannamei* larvae in previous studies. Pan (Pan et al., 2007) reported these findings; Lin (Lin et al., 2010) compared the tolerance of different families of *L. vannamei* to freshwater and obtained the basic population of excellent freshwater tolerant families; and Wang (Wang et al., 2022) discovered that both growth and comprehensive stress tolerance traits can be genetically improved through breeding, and growth traits can also be manipulated. This study aims to evaluate the stress tolerance of the breeding population, access the mortality rates of two introduced self-bred and hybrid families of *L. vannamei* under 96 h of high ammonia-N, high pH, and low salt stress, provide candidate materials for further family selection, and provide reference data for stress resistance breeding of *L. vannamei*.

Materials and methods

Test materials

The experiment was conducted at Hainan Lutai Marine Biotechnology Co., Ltd., using two introduced populations of *L. vannamei* as parent shrimp, namely the Dingfeng strain (T) from Thailand and the Daynight Express strain (M) from the United States. Each strain of parent shrimp is raised separately, with each female shrimp corresponding to an eye label. After the parent shrimp is temporarily raised and stabilized, the female shrimp undergoes unilateral eye stalk removal and is fed with frozen squid, oysters, and fresh sand silkworms to promote maturation. In order to maximize the success rate of mating, the female shrimp with large orange ovaries and the male shrimp with mature gonads were selected as parent shrimp. Four self-propagation and hybrid populations were successfully established within 10 days by selecting mature gonadal parent shrimp and using diallel natural mating: T♀ × T♂ (TT)、M♀ × M♂(MM)、T♀ × M♂(TM) and M♀ × T♂(MT), see supplementary (Table 1) for detailed parental information, a total of 20 families. The inseminated female had been moved back to individual spawning tanks before the spawned eggs were incubated in the spawning tank until hatching, with 2000 nauplii selected from each family, and their larval development was tracked and observed. Each self-breeding and hybrid population

TABLE 1 The biological data of parents.

parent		carapace length (mm)	body length (mm)	body weight (g)
T	♀	5.00 ± 0.27	18.04 ± 0.63	82.58 ± 7.94
	♂	4.32 ± 0.20	16.87 ± 0.59	62.18 ± 6.46
M	♀	4.72 ± 0.27	16.44 ± 0.66	63.42 ± 7.09
	♂	4.16 ± 0.23	16.06 ± 0.79	56.34 ± 6.47

was cultured in a standardized manner until the larvae were hatched. After cultivation to P_{15}, 1000 nauplii were randomly selected and transferred to the cement pool of the standard coarse workshop to maintain consistent cultivation conditions at all stages, and reduce the impact of differences in environmental conditions (mainly including salinity, temperature, larval density, feed, and inflation of water at different stages) on growth and development. When the body length of the juvenile shrimp had reached at 4-5 cm, larvae of similar sizes were randomly picked from the cages of each line to carry out the experiment. Three parallel shrimp were placed in each group, and 50 shrimp were placed in each parallel. Before the experiment, the juvenile shrimp specifications were: TT body length (5.11 ± 0.65) cm, body weight (1.34 ± 0.45) g/tail; MM has a body length of (4.99 ± 0.60) cm and a weight of (1.30 ± 0.44) g/tail; TM has a body length of (4.47 ± 0.50) cm and a weight of (0.90 ± 0.30) g/tail; MT body length (4.66 ± 0.55) cm, body weight (1.02 ± 0.37) g/tail. During the feeding period, the larvae were supplied every 2 h with a diet of microalgae, rotifer, Artemia salina, and compound feed (8.32% moisture, 48.65% crude protein, 5.70% crude fat, and 11.89% ash) until 24 h before treatment.

Test method

Before the start of the formal experiment, a 96-h tolerance pre-experiment was conducted to obtain the 96-h half-lethal mass concentration of *L. vannamei*, which includes high ammonia-N, high pH, and low salt stress concentrations of 130mg·L^{-1}, 9.7, and 2 ppt, respectively. The water in the experimental group was regulated with NH_4CL (analytically pure), NaOH, and fully aerated fresh water: temperature was maintained at 28 ± 1.5℃, dissolved oxygen content ≥ 5mg/L. 150 uniformly sized juvenile shrimp were selected from each family in the standard coarse pool for fluorescence labeling and temporarily raised for 24 h. Then 150 individuals were randomly divided into 3 groups, with 50 individuals in each group. They were mixed in three 7.3m × 4.3m × 2m cement ponds, respectively, and stress tests were carried out with high ammonia-N, pH, and low salt. During the experiment, there was no inflation, and individual deaths were observed at regular intervals. Dead individuals and feces were promptly removed, and the number of deaths within 96 h was accurately recorded.

Statistical analyses

Excel 2010 was used to calculate the average value, standard deviation, and heterosis of mortality for each family; SPSS26.0 was used to perform one-way ANOVA on the data; and Duncan's multiple comparison method was used to analyze the significance of differences between different combinations for traits with significant differences ($P< 0.05$). The results are expressed as mean ± standard deviation (mean ± SD).

We used the following heterosis formula for combined stress tolerance traits (Cruz and Ibarra, 1997; Lu et al., 2016):

$$H(\%) = \frac{\overline{F}_1 - \frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right)}{\frac{1}{2}\left(\overline{P}_1 + \overline{P}_2\right)} \times 100$$

Where $\overline{F_1}$, $\overline{P_1}$, and $\overline{P_2}$ represent the first generation of reciprocal offspring of parent 1 and parent 2, the mean value of inbred offspring of parent 1, and the mean value of the representative type of inbred offspring of parent 2, respectively; H (%) is the reciprocal offspring heterosis rate.

Results

Effect of stress tolerance traits on mortality of different *L. vannamei* families

The survival rates of 20 F_1 generation populations of juvenile shrimp under different ammonia-N, pH, and salinity stresses for 96 h are shown in Table 2. A one-way analysis of variance was conducted on the ammonia-N tolerance of each family, and the results showed that there were differences in the tolerance of different families to 96-h stress ($P< 0.05$). The range of acute stress tolerance mortality rates for 20 families under ammonia-N

TABLE 2 Effect of stress tolerance traits on mortality of different *L. vannamei* families.

Family	96h stress-resistant family mortality (%)		
	Ammonia-N	pH	Salinity
TT1	41.91 ± 12.89^{efghij}	42.38 ± 10.03^{ghi}	70.67 ± 15.01^{abcd}
TT2	19.52 ± 5.77^{k}	23.33 ± 7.87^{j}	61.33 ± 15.01^{bcd}
TT3	23.33 ± 9.28^{jk}	38.33 ± 6.01^{ghij}	73.33 ± 9.02^{abc}
TT4	73.34 ± 7.64^{bc}	92.22 ± 6.94a	77.33 ± 8.08^{ab}
TT5	53.89 ± 4.19^{def}	48.89 ± 15.75^{efgh}	70.00 ± 12.00^{abcd}
MM6	46.19 ± 5.77^{efgh}	33.33 ± 11.54^{hij}	72.67 ± 7.02^{abc}
MM7	25.00 ± 11.66^{ijk}	54.44 ± 9.18^{efg}	55.33 ± 9.02^{de}
MM8	35.90 ± 16.01^{fghijk}	39.49 ± 12.34^{ghij}	76.67 ± 11.02^{ab}
MM9	32.86 ± 6.55^{ghijk}	25.24 ± 5.77^{ij}	55.33 ± 8.08^{de}
MM10	51.28 ± 11.65^{defg}	54.36 ± 7.90^{efg}	19.33 ± 9.45^{g}
TM11	77.22 ± 5.09^{abc}	46.67 ± 7.64^{fgh}	67.33 ± 8.08^{abcd}
TM12	92.22 ± 12.06^{a}	83.63 ± 6.56^{ab}	60.67 ± 5.03^{cd}
TM13	67.88 ± 9.15^{bcd}	64.44 ± 8.39^{cde}	40.67 ± 3.06^{ef}
TM14	47.88 ± 12.38^{efg}	52.00 ± 8.00^{efg}	35.33 ± 6.11^{f}
TM15	81.11 ± 12.51^{ab}	80.00 ± 9.28^{abc}	80.00 ± 6.00^{a}
MT16	58.89 ± 8.22^{cde}	62.22 ± 5.09^{def}	40.67 ± 7.02^{ef}
MT17	28.33 ± 7.27^{hijk}	73.33 ± 11.11^{bcd}	70.67 ± 7.02^{abcd}
MT18	43.33 ± 16.42^{efghi}	73.89 ± 8.55^{bcd}	40.67 ± 3.06^{ef}
MT19	66.67 ± 9.02^{bcd}	71.11 ± 13.47^{bcd}	68.67 ± 5.03^{abcd}
MT20	34.44 ± 6.74^{ghijk}	33.33 ± 6.01^{hij}	70.67 ± 7.02^{abcd}

Values with different superscripts within the same row are significantly different from one another (P < 0.05), and those with the same superscripts have no significant difference.

stress for 96 h is 19.52% - 92.22%. The mortality rate of the TT2 family under ammonia-N stress for 96 h is the lowest, at 19.52% ± 5.77%, while the TM12 family has the highest, at 92.22% ± 12.06%. The range of tolerance mortality rate for acute stress families at pH 96 h is 23.33% - 92.22%, with family TT4 having the lowest survival rate and family TT2 having the highest survival rate; The MM10 family had the lowest acute stress tolerance death rate after 96 h of low salt tolerance, which was 19.33% ± 9.45%. The TM15 family had the highest mortality rate, which was 80.00% ± 6.00%. Under ammonia-N and pH stress, the TT2 family has the strongest tolerance. Some families also overlap in tolerance under pH and low salt stress. If the average mortality rate of a family is less than 30%, it is classified as a high tolerance family; 30% - 60% is a moderate tolerance family; and more than 60% is classified as a weak tolerance family; then the family coefficients of strong, medium, and weak ammonia-N tolerance are 4, 10, and 6, respectively. There are 2, 10, and 8 pH values, respectively; There are 1, 6, and 13 low salt levels, respectively.

Comparison of tolerance between different paternal and maternal lineages

Compare the mortality rates of families with different parental and maternal origins under different stress conditions at 96 h (Table 3). The results showed that under ammonia-N stress, the average mortality rate of families with a female parent from the United States was the lowest (42.29 ± 13.55%), but there was no significant difference compared to families with a male parent from Thailand or the United States ($P > 0.05$); Under pH stress, there was no significant difference in tolerance among families whose parents were originally from Thailand or the United States ($P > 0.05$), with the family with the strongest tolerance being the parent from the United States (52.07 ± 18.21%); Under low salt stress, the mortality rate of families with American parents was lower than that of families with American parents, while the mortality rate of families with Thai parents was lower than that of families with Thai parents, but there was no significant difference ($P > 0.05$).

Comparison of tolerance for families of different mate combinations

Duncan's multiple comparison method was used to perform variance analysis on the significant differences in mortality rates among four hybrid combinations under stress (Table 4). The results showed that under ammonia-N stress, the ammonia-N tolerance of the TM combination population was significantly lower than that of the other three combination populations ($P< 0.01$), and there was no significant difference in ammonia-N tolerance among the other three populations ($P > 0.05$); Under pH stress, the tolerance of self-pollinated population MM to pH was significantly higher than that of hybrid population TM and MT combination population ($P< 0.05$), while the tolerance of hybrid population TM was significantly lower than that of self-pollinated population, and there was no significant difference in pH tolerance between self-pollinated population and MT combination population ($P > 0.05$). Under low salt stress, there was a significant difference ($P< 0.05$) in the tolerance to low salt between the TT combination population and the MM and TM combination population, while there was no significant difference ($P > 0.05$) between the TT combination population and the MT combination population. The survival rate of the MM combination population was the highest under all three types of stress. The heterosis analysis of the mortality rate of inbred combinations and hybrid combination families from Thailand and the United States under stress resistance is shown in Table 4. The results show that the average heterosis rate of stress resistance of most combinations is positive, and all family combinations show different degrees of heterosis (14.89% - 81.67%) under Ammonia-N and pH stress, of which the TM combination shows high heterosis (81.67%, 44.58%) for Ammonia-N and pH tolerance. The hybrid generation showed a

TABLE 3 Comparison of tolerance between different paternal and maternal lineages.

Average mortality/%									
Origin of male parent	Number of family	Ammonia-N	pH	Salinity	Origin of female parent	Number of family	Ammonia-N	pH	Salinity
Thailand	10	44.37 ± 18.44	55.90 ± 21.93	64.40 ± 13.12	Thailand	10	57.83 ± 24.65	57.19 ± 22.18	63.67 ± 14.89
U.S.A	10	55.75 ± 22.65	53.36 ± 18.80	56.33 ± 19.55	U.S.A	10	42.29 ± 13.55	52.07 ± 18.21	57.07 ± 18.56

TABLE 4 Comparison of tolerance for families of different mate combinations.

	T × T	M × M	T × M	M × T	H(T × M, %)	H(M × T, %)	H(%)
Ammonia-N	42.40 ± 21.8^{b}	38.25 ± 13.46^{b}	73.26 ± 17.85^{a}	46.33 ± 17.26^{b}	81.67	14.89	48.28
pH	49.03 ± 25.91^{bc}	41.37 ± 12.92^{c}	65.35 ± 16.41^{a}	62.78 ± 17.12^{ab}	44.58	38.89	41.74
Salinity	70.53 ± 5.90^{a}	55.87 ± 22.64^{b}	56.80 ± 18.61^{b}	58.27 ± 16.09^{ab}	-10.13	-7.80	-8.96

certain degree of hybridization disadvantage (H (%)< 0) in low salt tolerance.

Survival rates of different populations under acute stress

Figure 1A showed that under ammonia-N stress, the survival rates of all four populations showed a significant downward trend at 48 h (P< 0.05), but the downward trend of the survival rates of the four populations was significantly different. Within 24 h of stress, the survival rate slowly decreased, and after 48 h, the TM population showed a rapid decline trend compared to the other three populations. Figure 1B showed that under pH stress, the survival rates of the four populations significantly reached a low point at 36 h (P< 0.05), and the decline rate slowed down thereafter. The self-crossing populations TT and MM, as well as the hybrid populations TM and MT, maintained a consistent downward trend; Figure 1C showed that under low salt stress, the survival rate of the four populations showed a rapid decline at 12 h, and thereafter, the

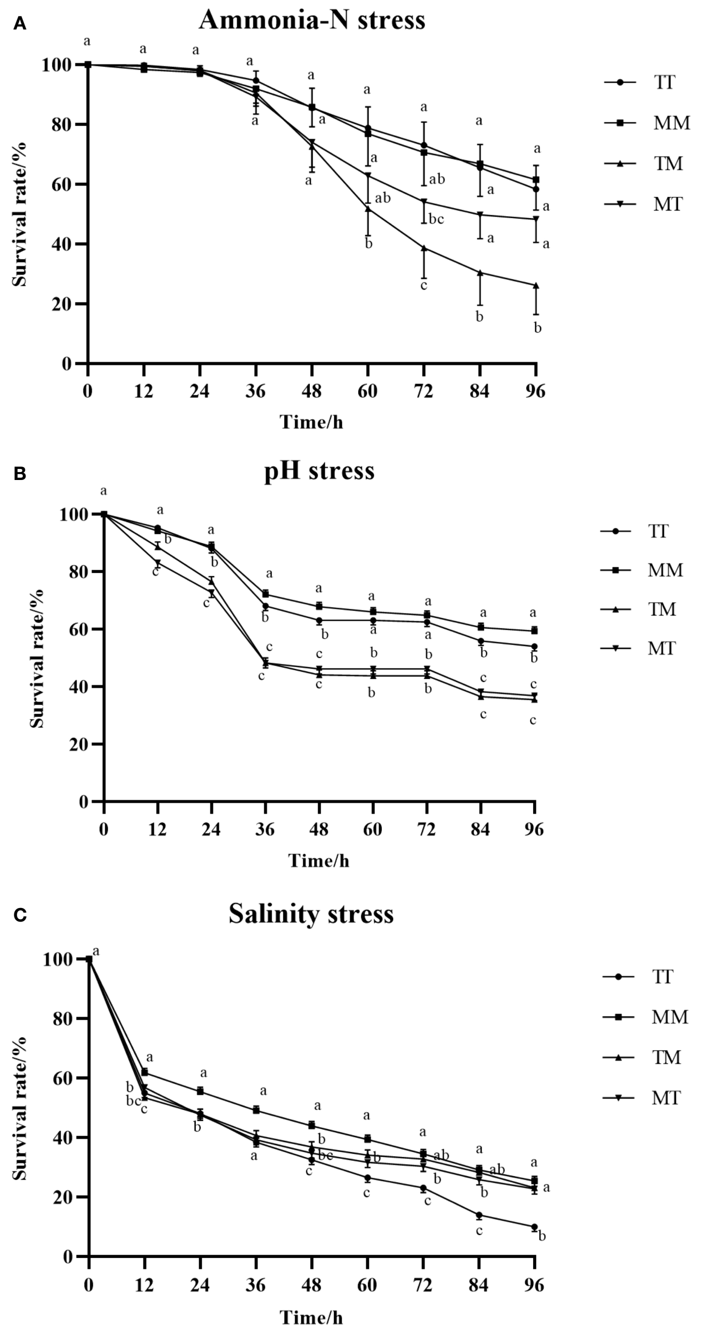

FIGURE 1
Survival rates of different populations under acute stress (Ammonia-N stress **(A)**, pH **(B)** stress, Salinity stress **(C)**). The lowercase letters represent each of the line graphs in the figure.

decline trend of the four populations slowed down, and there was no significant difference in survival rate between the four populations ($P > 0.05$).

Discussions

The genetic basis, disease resistance and pathogenicity of pathogenic microorganisms of mariculture animals are significantly impacted by adverse water quality factors in the aquaculture environment, such as abnormal temperature, salinity, ammonia-N content, pH, nitrite, and other stress factors (He et al., 2008). The main pollutant in the environment for shrimp farming is Ammonia-N, which is primarily found in the water body as non ionized ammonia (NH_3) and ionic ammonia (NH_4^+), both of which are in a dynamic equilibrium. Because NH_3 is not charged and has high fat solubility, it can penetrate cell membranes and negatively affect shrimp growth, molting, oxygen consumption, immunity, ammonia-N excretion, osmotic pressure regulation and disease resistance (Lei and Chen, 2003; Huang et al., 2012; Cui et al., 2017). pH is an important ecological factor that affects the survival status of aquaculture organisms and can reflect the quality of the water. Due to their extreme sensitivity to acidification and alkalization, shrimp and crab species can be somewhat impacted by high or low pH levels in terms of growth, immunity, ion balance, etc. The osmotic regulation of crustaceans is closely related to environmental salinity. Acute salinity stress will have an impact on the osmotic pressure of the internal environment, the level of respiratory metabolism, and immune function (Camacho-Jiménez et al., 2018; Joseph and Philip, 2020). Breeding work based on family lines, such as *Pinctada martensii*, *Argopectenirradians*, *Patinopectenyessoensis*, *Paralichthys olivaceus*, etc., has gradually become more popular in recent years (He et al., 2007; Qin et al., 2007; Chen et al., 2008; Zhang et al., 2008). When compared to population selection, family selection has the advantages of fast speed, obvious effects, high work efficiency, flexible operation, and outstanding excellent traits. The family's genetic composition is relatively uniform, making it simple to determine the genotypes of the parents and offspring. As a result, family selection has begun to be widely applied in the breeding of superior aquatic animal varieties. Another important advantage of family selection is the ability to estimate the breeding values of some difficult to measure traits of the parents by observing the phenotypic traits of whole or half sib families. This enables the selection of quantitative traits through family selection, such as growth rate and disease resistance (Wu et al., 2011).

Twenty families from 4 populations of 2 introduced populations of *L. vannamei* were used as experimental subjects in this study through a combination of self-breeding and hybridization. Seven families with strong tolerance were initially selected using ammonia-N, pH, and salinity tolerance as indicators. The optimal families under ammonia-N and pH stress overlapped (TT2), and some families overlapped under low salt and pH stress. Chinese shrimp larvae from different families were subjected to acute toxicity tests, and He (He et al., 2008) discovered that there were significant differences in ammonia-N tolerance and high pH traits, indicating significant selection potential. Chen (Chen et al., 2017) tested the low salt tolerance of 11 families of *Penaeus monodon* and found significant differences in each family's survival rate at half-lethal time. In order to select strains of shrimp with stress resistance and resolve the paradox between environmental degradation and high yield in the later stages of shrimp breeding from the perspective of genetic improvement of breeding varieties, comparative analysis of physical differences between different populations, further analysis of heterosis of stress resistance, and comparison of genetic differences between different mating combinations are necessary (Camacho-Jiménez et al., 2018). In this experiment, a study on the mortality rate of families constructed from the same source of maternal and paternal parents found that although the mortality rates of families were different, there was no significant difference ($P > 0.05$). The progeny populations whose male parent were from the Thai and American populations had higher survival rates than those whose female parent were from the Thai and American populations under ammonia nitrogen and pH stresses, but the opposite results were observed under low salt stresses. The overall tolerance of different groups of families to ammonia-N, pH, and low salt is generally higher than that of Thai sources. The American strain (MM) and a few hybrid strains (MT) performed better under ammonia-N, pH, and low salt stress, whereas the Thai strain (TT) and a few Thai American hybrid families performed worse, indicating the American strain's advantages in stress resistance. It can be assumed that this advantage was brought about by the American strain's strong capacity for stress resistance. The Thai strain is relatively weak, and another possibility is that the advantage of stress resistance is determined jointly by both hybrid strains rather than by the advantage of a single strain. The possibility needs to be further investigated in future generations. The analysis showed that the hybrid families of Thailand and the United States had heterosis for ammonia-N and pH tolerance, and that, with the exception of low salt stress, the average heterosis value of growth traits of hybrid combinations was positive (14.89% to 81.67%). *L. vannamei*'s growth traits have been the subject of many studies both domestically and abroad, and it has been discovered that hybrid combinations generally show heterosis in growth performance (Sui et al., 2016; Lu et al., 2017). For ammonia-N and pH traits, TM and MT populations showed different levels of heterosis, but overall, the heterosis of TM was better than that of MT, and the average heterosis rate of the TM population for ammonia-N was the highest, 81.67%. In low salt tolerance, the hybrid combinations showed a certain degree of hybridization disadvantage (−10.13% to −7.80%), which was weaker than the median of both parents. Ji (Ji et al., 2018) compared the stress resistance and growth traits of the first generation of shrimp in different populations of *L. vannamei*, and found that the results were consistent. The survival rates of the four populations under acute stress showed that the stress resistance of the MM and MT populations was slightly higher than that of the TT and TM populations, and that the inheritance of the maternal parent was dominant. The hybrid MT population demonstrated significantly greater stress resistance than that of the TM population. Different cross combinations show different heterosis, and heterosis of different characters also varies. Heterosis is a complex biological phenomenon. According to some studies, the stronger the complementarity and the more obvious the heterosis, the greater the genetic difference between hybrid parents (Pillai et al., 2011). In this experiment, the hybrid population showed poor performance in low salt stress resistance, which may be due to the enrichment of harmful recessive genes at different loci in parents with different genetic backgrounds. The probability of growth or stress resistance decline in the hybrid offspring will increase if the harmful recessive genes in the chromosomes provided by the parents of the hybrid offspring happen to be alleles (Ma et al., 2005). Selection of parents is crucial in the hybrid breeding process because improper parent selection could result in the regression of certain important economic traits in hybrid offspring.

Stress resistance is a threshold trait that multiple genes control and refers to an organism's ability to adapt to harsh environments. It is generally believed that heritability is relatively low and that it is relatively difficult to improve traits through breeding (Bulmer, 1980). The research on the stress resistance of *Penaeus monodon* (Huang et al., 2012), *Macrobrachium rosenbergii* (Thanh et al., 2010) and *Penaeus chinensis* (Li et al., 2005) shows that the stress resistance of some aquatic animals has high heritability and can be improved through breeding. This study screened out superior populations (MM and MT), as well as high ammonia-N and high pH families (TT2) and low salt families (MM10) using the average mortality rate and hybrid advantage of families under high ammonia-N, high pH, and low salt stress for 96 h. The results of this study provide two candidate dominant families for the breeding of *L. vannamei* families. The next step is to preserve the selected families with strong stress resistance and improve the stress resistance of the families through continuous breeding and purification. In addition, the genetic structure of different populations and families will be analyzed at the molecular level, and molecular marker-assisted breeding will be carried out for further verification and selection in future breeding work. To provide reference data for breeding a new strain of *L. vannamei* that is fast-growing and stress-resistant and has significantly improved target economic traits. to.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of the South China Sea Fisheries Research Institute.

Author contributions

MS, SJ, YL, and FZ: conceptualization. MS, SJ, SGJ, YL, and QY: methodology. MS and SJ: data curation. MS: writing—original draft preparation. FZ: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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