doi: 10.7541/2023.2022.0044

B “ 3 ”

1 1 1 1 1, 2

(1. , , 266003; 2. , , 266237)

: B(CB) “ 3 ” , CB (0.2 0.4 0.6 0.8 mg/L) (6 8 10 12) (10 15 20 25min) , , CB 0.6 mg/L, 15min , (65.69±2.47)%, ; 8, 15min , (38.77±2.69)%, CB , , CB (*P*<0.05), CB (14.2±1.08) (10.49±0.60) μm/d, (15.43±1.08) μm/d; 2 , CB , CB , CB , 12d , “ 3 ”

: ; B; ; ;
: Q172 : A : 1000-3207(2022)00-0001-08

(*Crsssostrea gigas*) , , [5] , , , , , 2020 , [6] , 132.33×10^7 kg, 8.04 [1] 100% [7] , [8] Guo [9] , , “ 3 ” , , , [2] , “ 3 ” B(CB) 6- [9—12] (6-DMAP) , CB , [13] (,) [3], , , [14]; 60% , , [15] , , [4] , [9, 16]

: 2022-01-26; : 2022-04-18
: (CARS-49); (2020LZGC016) [Supported by the China Agriculture Research System Project (CARS-49); the Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (2020LZGC016)]
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,

“ 3 ”

, CB “ 3 ”

,

, “ 3 ”

1

1.1

2021 5 1 “ 3 ”

, “

3 ” 2020 6 “ 3 ”

CB

,

(1A 1B)

1.2

“ 3 ”

“ 3 ” , B

CB

CB ,

5×10^7 egg/L, 4 , ,

, 4

CB (0.2 0.4 0.6 0.8 mg/L)

15min, , 1 mL/L

(DMSO) CB;

, CB 0.6 mg/L 10

15 20 25min 4 (6

8 10 12),

15min;

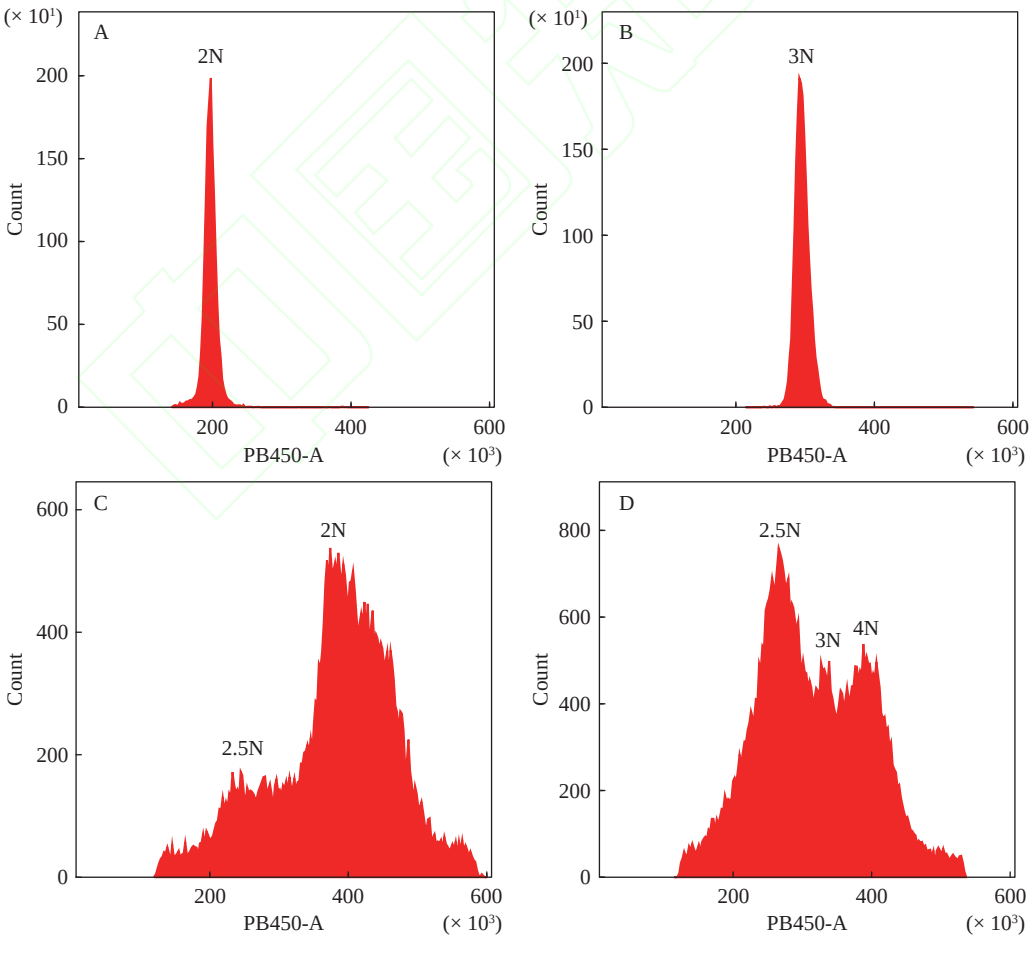
, 8 10 15 20

25min (24±1) 29±1

, 3

1.3

24h , 300 ,



1 DNA

Fig. 1 Relative DNA content and ploidy level of parents and each experimental group by the flow cytometry

A. ; B. ; C. CB ; D.

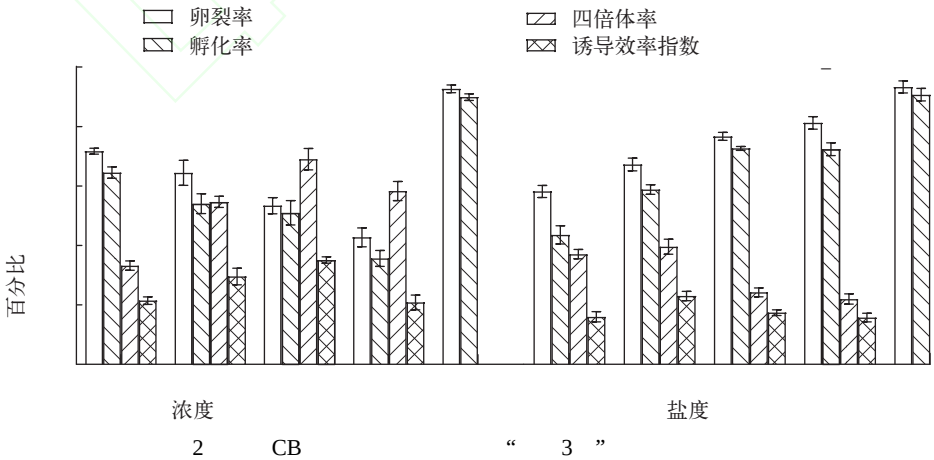
A. Diploid oyster; B. Triploid oyster; C. CB group; D. Low salinity group

70 L , CB 0.6 mg/L
1×10^4 ind./L 3 , (69.02±2.94)% (1C),
(*Isochrysis galbans*), (0.351) , 0.4 0.8 mg/L
(*Platymonas subcordigoramis*), (*P*<0.05; 2A)
, (24 ± 1) CB “ 3 ”

1.4 0.6 mg/L
4h 2d 4d 6d 8d 10d 12d , , “
, 3 ” ,
4h , 8 ,
D , (39.10±2.03)% (1D), (0.227)
, , 6
[17] (*P*>0.05), (*P*<0.05;
D 2B), 10
1.5 mL , 1.5 mL (*P*<0.05)
3—6 , 500 “ 3 ” 8
75% 1 mL DAPI (2 μg/mL) **2.2** **“ 3 ”**
75% , ,
CB , ,
2n “ 3 ” ,
± ,
SPSS 25.0 15min , (65.69±2.47)%,
(ANOVA) (Duncan) , (0.363) , 10min
P<0.05 (*P*<0.05),
(*P*>0.05),

2

2.1 CB “ 3 ” 20min
(*P*<0.05; 3A) CB “
CB , CB , “ 3 ” 15min
, , ,
,



2 CB “ 3 ”

Fig. 2 Effects of different CB concentrations and salinities on tetraploid induction of the Pacific oyster “Haida No. 3”

A. CB ; B. ; CG. ; (*P*>0.05),
(*P*<0.05),

A. CB group; B. Low salinity group; CG. control group. The same indicator with the same letter means that the difference between groups is not significant (*P*>0.05), and with different letters means that there is a significant difference between groups (*P*<0.05). The same applies below

15min , (38.77±2.69)%, (0.233) , 10min (*P*<0.05), (*P*>0.05), 20min (*P*>0.05), (*P*<0.05; 3B) “ 3 ” 15min

2.3 “ 3 ”

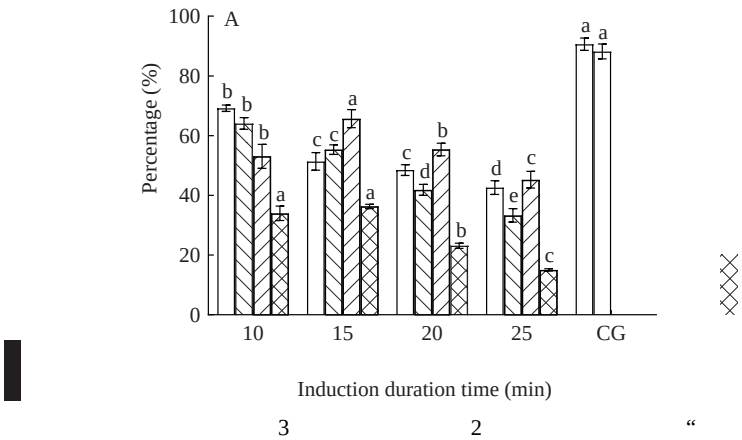
, CB , , CB (*P*<0.05) 12 , CB (170.39±12.99) μm, (125.86±7.2) μm, (185.20±12.9) μm 2 CB (*P*<0.05); 4 CB (*P*<0.05), (*P*>0.05); 6 CB , CB (*P*>0.05), (*P*<0.05), CB (*P*<0.05); 8 10 >CB > , (*P*<0.05); 12 CB (*P*<0.05), CB (*P*>0.05; 4) , 2 , CB , CB 12 , CB (12.35±5.02)%, (3.74±1.62)%, (29.00±6.48)% 2 > >CB , (*P*<0.05); 4—6 , 6 CB , (*P*>0.05), CB (*P*<0.05); 8—12 , >CB > , (*P*<0.05; 5)

2.4 “ 3 ” , , , , “ 3 ” CB 12d , CB “ 3 ” (1)

3

3.1 “ 3 ”

1981 Stanley [18] CB , , CB [9, 16, 19] , “ 3 ” CB 0.6 mg/L, , , CB , (PB1) , ; CB

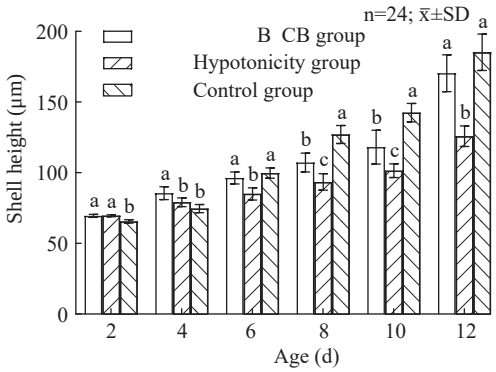


3 2 “ 3 ”

Fig. 3 Effects of two induction methods on tetraploid induction of the Pacific oyster “Haida No. 3” at different durations

CG. ; A. CB ; B.

CG. control group; A. CB group; B. Low salinity group



4 “ 3 ”

Fig. 4 Effects of two tetraploid induction methods on the larvae growth of the Pacific oyster “Haida No. 3”

5 “ 3 ”

Fig. 5 Effects of two tetraploid induction methods on the larvae survival rate of the Pacific oyster “Haida No. 3”

1

Tab. 1 A comprehensive comparison of the effects of the application for two tetraploid induction methods

Induction method		CB	Low salinity
Toxicity			
Operability			
Cleavage rate (%)		40.60~71.69	53.16~80.31
Hatching rate (%)		33.30~64.51	43.06~71.83
Tetraploid rate (%)		33.23~69.02	27.54~39.10
The efficiency of tetraploid induction		0.150~0.363	0.125~0.233
12d	12-day survival rate (%)	12.35±5.02	3.74±1.62
	Growth rate (μm/d)	14.2±1.08	10.49±0.60
	Cost (/mg)	800	

, , ,
[9, 16, 20]
, Barreto [21] CB(1 mg/L)
(*Argopecten nucleus*) , ,
21%, 9

Bruce [22] CB(0.25 mg/L)
,
,
, ,
[15]
,
“ 3 ” 8,
,
6 8 ,
(P>0.05), 6 ,
,
[23] 8
, ,
,
,
[12, 16]
,
, “ ” “
” “ ” “ ”
,
“ „[24]
,
[25]
,
, ,
,
, 15min
[23, 26]
, ,
[27, 28]
,
,

3.2 “ 3 ”

, CB
, , CB
(P<0.05) Richard [29]
CB 6-DMAP ,
Yang [30]
CB

CB ,
CB [21] , CB
mRNA
,
(mRNA) , CB

[31] Meng [32]

“ ”,

,

,

, CB

, CB

, 4—6 ,

CB :

(1) 48h ,

[27, 33] (2)

[34] (3)

[28]

[30, 35]

3.3 “ 3 ”

[10, 11] CB 6- (6-

DMAP) [9, 12],

CB “ 3 ” , ,

, ,

, , ; CB

,

, ,

CB ,

“ 3 ”

:

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TETRAPLOID INDUCTION OF THE PACIFIC OYSTER “HAIDA NO. 3” USING CYTOCHALASIN B AND LOW SALINITY

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Abstract: To investigate the optimal conditions for the tetraploid induction of the Pacific oyster *Crassostrea gigas*

“Haida No. 3” by cytochalasin B (CB) and low salinity, the effects of CB concentration (0.2, 0.4, 0.6 and 0.8 mg/L), low salinity (6, 8, 10 and 12) and induction duration (10, 15, 20 and 25min) on the cleavage rate, hatching rate, tetraploid rate and the efficiency of tetraploid induction were estimated by inhibiting the first polar body of fertilized eggs. At the same time, the growth and survival of the larvae were analyzed. The results showed that the maximum point of tetraploid rate (65.69±2.47)% and the efficiency of tetraploid induction was found at CB concentration of 0.6 mg/L and induction duration of 15min. In low-salt induction, the maximum point of tetraploid rate (38.77±2.69)% and the efficiency of tetraploid induction was found at salinity of 8 and induction duration of 15min. The shell height of CB and low-salt treatment groups were larger at the early stage and smaller at the later stage compared with the control group. The shell height of the CB treatment group was significantly greater than the low-salt treatment group (P<0.05), and the mean daily growth of larvae in the CB and the low-salt treatment groups was (14.2±1.08 μm/d) and (10.49±0.60 μm/d), respectively, which were smaller than the control group (15.43±1.08 μm/d). The survival rate of the two induction treatment groups was consistently lower than the control group, and the survival rate of the low-salt treatment group was higher at the early stage and lower at the late stage compared with the CB treatment group. In general, the CB induction method showed better results in terms of tetraploid rate, the efficiency of tetraploid induction, 12-day survival rate and growth rate, and has better applicability for the tetraploid induction of the Pacific oyster “Haida No.3”.

Key words: Tetraploid rate; Cytochalasin B; Low salinity; The efficiency of tetraploid induction; *Crassostrea gigas*