Study on the breeding technology of heterosis seedling of *Laminaria* and new combinations

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Abstract
After continuous breeding gametophyte of *Laminaria*, then breaking protonema, heterosis seedling is got by bilineal hybridization. The result shows that the weight-increasing rate of female and male gametophyte clone cells are 14.03% and 13.87%, after 16 d crossbreeding, the rate of ovulation is up to 100%; the length of juvenile sporophyte after 40 d breeding is 2–3 cm. By farming on the sea, a hybrid combination is primarily screened.

Key words: *Laminaria*, gametophyte clone, bilineal hybridization, hybrid vigor

1 Introduction

Hybrid vigor is in normal existence in nature. Its offspring has more advantages in yield, resistance and adaptability, which receives people’s attention. Over 80% species of crops in the world are hybrid vigor and they bring huge economic benefits.

The base work for breeding by using hybrid seeding and hybrid vigor has begun since the 1970s. Fang et al. (1979) has successfully bred the gametophyte clone of *Laminaria*, and pointed out that this clone has the characteristics of sex differentiation, phyad purity, totipotency of development and so on. This can provide necessary materials to utilize the advantage of hybrid vigor. Also they have reported the work by using this advantage (Ou et al., 1983; Fang et al., 1982). During the farming of *Laminaria*, the hybrid species of Danza 10, Yuanza 10 and 901 are popularized, which contributes greatly to the high quality of *Laminaria*.

During 1999–2000, the authors did hybrid vigor seedling by bilineal hybridization. The work is reported as follows.

2 Materials and methods

2.1 Materials

Gametophyte clone cell of *Laminaria* is provided by the Germ Glasm Center, Ocean University of China.

2.2 Methods

2.2.1 Culture requirements

Amplification requirement for gametophyte clone cell of *Laminaria*: 8–12 °C; 60 μmol/(m²·s); 12L: 12D photoperiod; culture medium: sterile seawater, nutrient saline (NO₃ — N 16 mg/
L, PO₄ — P 1 mg/dm³; culture with aeration all along.

2.2.2 Condition for smashing clone cells

Ultrasonic cell disintegrator YJ92–2, ice-bath (Liu et al., 2000).

2.2.3 Bilineal hybridization

Choose clone cells of female and male gametophyte from different germ lines, and mix together according to some proportion, and then make seedling attachment.

2.2.4 Seedling attachment

Use palm hangings (15 cm×20 cm each) as attachment base. With ultrasonic cell disintegrator, gametophyte clones are smashed to make into cell suspension, then sprayed to palm hangings. After 24 h attachment, wash off the unattached ones by culture medium, then make the palm hangings to be cultured under the condition as the same for clone cell development.

2.2.5 Breeding

The requirement is the same as that for gametophyte clone cell development needed. Remove Fe²⁺ from culture medium after gametophytes grow up to sporophyte. Aerate all along, and replace culture medium every 48 h.

2.2.6 Farming experiment

Follow the technology standard of Laminaria farming.

3 Results and discussion

3.1 Amplification culture of gametophyte clone cell

3.1.1 Experiment on seed quantity comparison (Table 1)

In microbiology fermentation and microalgae continuous culture, the original density is important to growth. According to this experiment, the original density of 0.500–1.000 g/dm³ can get better amplification effect (Table 1).

3.1.2 Experiment on continuous culture of female and male gametophyte clone cells

With the rate of 1 g/dm³, the experiment on continuous culture of female and male gametophyte clone cell was taken during August 23 to October 4, 1999. The result is shown in Fig. 1.

As Fig. 1 shows, the average increasing weight of female clone is 0.280 6 g/d, the aver-
age growth rate is 14.03% per day, and the growth peak appears at the 28th day; the average increasing weight of male clone is 0.2767 g/d, the average growth rate is 13.87% per day, and its growth peak is at the 21th day. Both of their peak appear in a period of 20~30 d. When the experiment finishes, the fresh weights of clone cells show little difference.

3.2 Experiment on bilineal hybridization of gametophyte clone of Laminaria (Table 2)

As Fig.2 shows, the development of bilineal hybridization group is advanced for 7 d according to female parthenogenesis group, and has more rapid growth and better synchronism. Bilineal hybridization group can get to the peak after the 28th day of culture, and its ovulation rate is 95.5%; while the female parthenogenesis group’s development rate is only 3.4%. This may relate to the sexual inducer which is produced after the female and male gametophyte clones mix together.

From Fig.2, it can be seen that the 2:1 rate between the female and male gametophyte clone cells during the growth peak period can have the biggest influence on the ovulation rate, while the rate of 1:10 can have the least one. Moreover, we find that the influence is continuously increasing with the rate from 1:10 to 2:1. On April 17, that is, the 7th day after the experiment began, female gametophyte had entered the ovulation period. On April 20, i.e., the 10th day, with the rate of 2:1 between female and male gametophyte, the ovulation rate was up to 100%. Until April 29, each experiment group reached the peak. By variance analysis, $P=2.81 \times 10^{-4}$, each group has significant difference.

The development ways of Laminaria gametophyte are 2: egg-sperm binding and unisexual reproduction (female parthenogenesis by only female gametophyte; male gametophyte can take autogeny by itself). When female or male gametophyte cell develops into egg sac or gonecyst, each one will become a reproductive cell. In nature, swarm spore coming from sporange reduction division develops into female and male gametophyte which rate is nearly 1:1. This pro-

![Fig. 2. Synchronous development comparison between female and male gametophyte clone of Laminaria.](image)

Table 2. Experiment on development comparison between the female parthenogenesis group and the bilineal hybridization group

<table>
<thead>
<tr>
<th>Group</th>
<th>Oct.30</th>
<th>Sep.3</th>
<th>Sep.6</th>
<th>Sep.9</th>
<th>Sep.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female parthenogenesis(%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Bilineal hybridization(%)</td>
<td>1.2</td>
<td>39.2</td>
<td>76.4</td>
<td>80.4</td>
<td>95.5</td>
</tr>
</tbody>
</table>
cess is not influenced by plant hormone (The data will be submitted later). During the growth (about 14 d), the female gametophyte has 1–2 cells (mostly just 1 cell), while the male gametophyte has 3–5 cells which can ensure the female one to be fertilized. From the experiment data, it is found that when the female and male gametophyte rate is 2:1, most eggs will not be fertilized. To assure young sporophytes of becoming hybrid *Laminaria*, the rate should be at least 1:1. In this experiment, when the gametophytes’ rate for hybridization is 1:1, the ovulation rate is up to 100% after 16 d in laboratory, and this can fit the need of breeding hybrid vigor seedling of *Laminaria*.

As shown in Fig.3, the growth speed of

![Graph showing ovulation rate (%) for *Laminaria* species and cross breed](image)

Fig.3. Sea-farming data of bilineal hybridization seedling and cultural species of *Laminaria*.

*Laminaria japonica* is fast with a developing rate of 77.29% after 10 d culture, while the *Laminaria japonica* developing rate in formal period is lower than the hybrid group, and higher in later period. We make variance analysis for the development rate of *Laminaria japonica* gametophyte seeding and clone bilineal hybridization, *P*=0.327 6, and no significant difference is found.

3.3 Sea-farming of bilineal hybridization seedling

Bilineal hybridization seedling was farmed on the sea during October 2000–June 2001. The results are as follows (Table 3).

Comparing several economic characteristics with *Laminaria japonica* and culture species, it can be seen that Crossbreeding 1 has some advantages in leaf length and fresh weight, and Crossbreeding 2 has better potential in leaf depth. Choosing the hybrid parents is one of the keys to breeding vigor seedling by bilineal hybridization. The correlated experiments need further investigating.

4 Conclusions

Recently, seedling and breeding of *Laminaria* are still based on sporophyte stage. Species intermixing, characteristics degeneration and rising cost for culture are no longer adapted to the requirement for modern aquiculture development. Using gametophyte clone not can only assure quality, cut cost, and shorten cul-

<table>
<thead>
<tr>
<th>Groups</th>
<th>Leaf length/cm</th>
<th>Leaf width/cm</th>
<th>Middle width/cm</th>
<th>Leaf depth/cm</th>
<th>Fresh weight/kg</th>
</tr>
</thead>
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<tr>
<td>Crossbreeding 1</td>
<td>450</td>
<td>34.5</td>
<td>8.5</td>
<td>2.3</td>
<td>1.92</td>
</tr>
<tr>
<td>Crossbreeding 2</td>
<td>310</td>
<td>40.5</td>
<td>8.0</td>
<td>3.0</td>
<td>1.46</td>
</tr>
<tr>
<td><em>Laminaria japonica</em></td>
<td>315</td>
<td>41.5</td>
<td>8.0</td>
<td>2.0</td>
<td>1.22</td>
</tr>
<tr>
<td>Cultural species</td>
<td>305</td>
<td>39.0</td>
<td>9.6</td>
<td>2.4</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Table 3. Sea-farming data of bilineal hybridization seedling and cultural species of *Laminaria*
ture period, but also reduce the appearance of disease during the traditional farming (Liu et al., 2000; Liu et al., 2000; Li et al., 2003). Now this new technology is combined with traditional breeding: separately bring up large numbers of female and male gametophytes; control their development; and hybridize to become sporophytes of Laminaria with excellent characteristics. Furthermore, these hybrid seedlings of the first final generation have the same heredity base, so we can utilize hybrid vigor to achieve high yield and quality.

Acknowledgements

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References