INTRASPECIFIC CROSSINGS OF *UNDARIA PINNATIFIDA* (HARV.) SUR.
——ON MORPHOLOGICAL AND GROWTH VARIBILITY OF JUVENILE SPOROPHYTES*

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Abstract Unicellular gametophyte clones of *Undaria pinnatifida* were isolated in Tokushima, Japan, and Qingdao, China in April—June of 1995. Vegegative gametophyte clone cultures were obtained and multiplied. In 1996, various gametophytic clones were used to complete different intraspecific crossings in lab. In each of the tries, juvenile sporophytes (0.8—1.5cm in length) were sucessfully obtained. Morphological features and growth rate were analysed. Morphological and growth variances among juvenile sporophytes resulting from different crossing combinations occurred at very early developmental stages, which are attributable to genetic differences. Juvenile sporophytes resulting from the same crossing combination of unicellular parental male and female gametophytes, in each case, possessed highly identical morophology and growth rate. This result is in accordance with previous observations on adult sporophytes: offsprings from the same crossing combination of unicellular gametophytes possessed uniquely identical morophology after months' open sea cultivation. Further, evidences show that selection of strains that have desired morphological characteristics and growth potential by screening unicellular gametophytes is highly possible. This might be a new way for seedling production and strain selection of this economically important brown macroalga.

Key words *Undaria pinnatifida* Intraspecific crossing Gametophyte

Subject classification number Q32

Comparative studies on morphological variances, growth capacity, contents of certain kind of substances, such as alginic, idone of adult sporophytes resulting from either intraspecific of interspecific hybridization were very well conducted in the past decades (Bolton *et al*, 1983; Chapman, 1974; Lewis *et al*, 1994; Lüning *et al*, 1978; Migita, 1967; Saito, 1972; Sanbonsuga *et al*, 1978). The improtance of these studies not only lies in that many problems related to traditional taxonomy, geographical distributation on the evolutionary point of views could be possibly clarified, but also in the fact that strains which possess desired characteristics can be probably obtained, which will in turn benefits seaweed production. Previous intraspecific crossing study of *U. pinnatifida* showed that some morphological features of adult sporophytes were independent of environmental

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conditions and sporophytes had sigificantly varied growth potential (Saito, 1972; Pang *et al*, 1997). In this paper, the results of an analysis of morphological and growth variances of juvenile sporophytes resulting from different combinations of unicellular gametophytes were presented, which are well in accordance with previous observation that sporophytes resulting from uni—combination of parental gametophytes possess highly identical morphological features.

1 Materials and Methods

1.1 Isolation and multiplication of gametophytes

Unicellular male and female gametophytes isolated from one adult sporophyte of *Undaria pinnatifida* in Tokushima, southern Japan in March 1995 were vegetatively propagated under the condition of 23℃, less than 5 μmol / (m^2 • s) dim light, L:D = 16:8, in VS medium. (von Stosch's enriched seawater). The isolation and propagation precedures were that, (1) zoospores were released into enriched sea water (PES, Provasoli, 1968) by using traditional method; (2) zoospore solution was greatly diluted to the density of 2—5 per field (100 ×) under microscope; (3) sildes with seeded zoospores were cultured in 20℃ for one month in dim light until the small clones of gametophyte were visible and well-distributed under microscope; (4) clones of unicellular gametophytes were then picked up by a sterilized pipette one by one under microscope; and (5) unicellular gametophytes were vegetatively propagated under the environmental condition described above. In case of faster multiplication of gametophytes, an electric blender (Ultra-Turrax T 25, Janke&Kunkel GMBH& Co. KG) was used to break down macro-gametophyte into shorter filaments (22 000 r / min) for several minutes depending on the size of the unicellular gametophyte clones) (Perez *et al*, 1984; Pang *et al*, 1996).

1.2 Inoculation procedures

Vegetative male and female gametophytes cultured in 16:8 light-dark regime were broken down to short filaments (6—15 cells for female, 10—30 cells for male), mixed together, and seeded on slides. Gametophytes seeded on slides were then cultured in 23℃, L:D = 12:12, irradiance 25μmol / (m^2 • s), in VS medium for two weeks until juvenile sporophyte were visible and then transferred into culture vessels (2 L in volume) and continously cultured in 15℃, L:D = 16:8, 25μmol / (m^2 • s),in VS medium with strong areation.

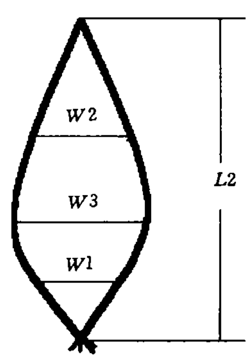

Fig.1 Morphological parameters of sporophytes of *U. pinnatifida*. (*W1*, *W2*, *W3* indicate different widths; *L2* represents frond length)

1.3 Measurements of morphological characteristics and growth rate of juvenile sporophytes

15—20 days after seeding on slides, juvenile sporophytes were visible and then transferred into 2L beakers, cultured in the VS medium with strong babbling. When reaching 0.8—1.5cm in length, morphological parameters of the juvenile sporophytes were obtained on the basis of Fig. 1. For each combination, ten sporophytes were sampled. Each of the length and width is the average value of the plants. Frond area of juvenile sporophytes were measured using a computer-aided bioimage system.

2 Results

The juvenile sporophytes resulting from the same crossing combination of parental unicellular gametophytes showed highly identical morphological characteristics as illustrated in Fig. 2 and Fig. 3.

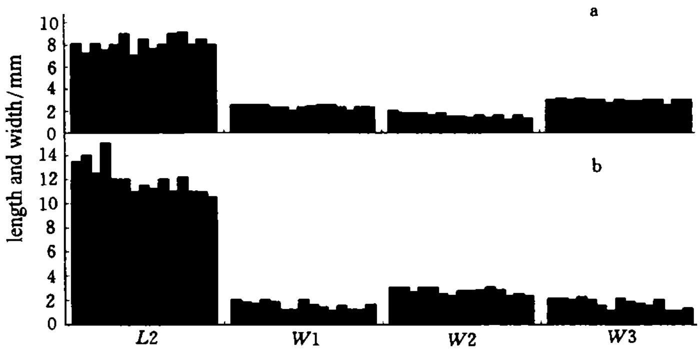

Fig. 2 Comparison of morphological features of sporophytes resulting from two crossingn of *U. pinnatifida* (Fifteen individuals for each crossing, showing that juvenile sporophytes inside one crossing possess identical morphological features. a: D1+D2; b: D2+D8. For explanations of *L2*, *W1*, *W2*, and *W3*, see Fig. 1)

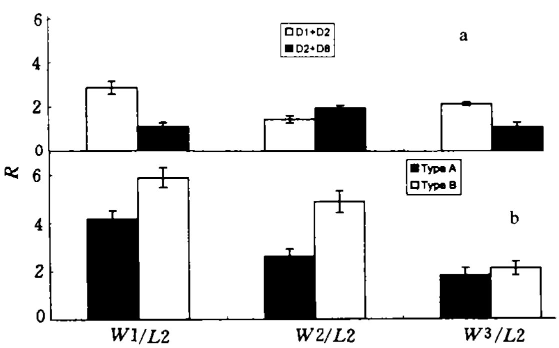

Fig. 3 Comparison of different morphological ratios of juvenile sporophytes of *U. pinnatifida* (a. juvenile sporophytes of two crossings with the same female parental gametophyte (D2) and different male parental gametophytes; b. two distinctly different juvenile sporophytes resulting from crossings of six strains of unicellular gametophytic clones. For other details, see Materials and Methods)

The parental unicellular gametophytes D2 (female) and D1, D8 (male) were isolated in Tokushima, Japan. Juvenile sporophytes with the same length had little variance in *W1*, *W2*, *W3*, and *L2*. Juvenile sporophytes of two crossings, though the female parental gametophyte was the same one, had different morphological features. Markedly higher value of W2 was recorded in D1 + D2 compared with another cross D2 + D8.

The reason to use *W1*, *W2*, and *W3* to compare the morphology of juvenile sporophytes is that the morphological charcteristic of the young plant can be well represented in this way. From Fig. 2 and Fig. 3, the offsprings from the same crossing combination possessed identical morphology, whilst the offsprings from different crossing combinations had variable morphology. More results shown in Fig. 4 further proved support to this point.

Furthermore, another crossing try was undertaken using six different unicellular gametophyte clones.In this cross, two kinds of juvenile sporophytes, which showed distinctly different morphological characteristics, were obtained (Fig. 3). When the six different unicellular gametophytes were combined and crossed together, at least three morphologically distinct juvenile sporophytes were obtained, of which two are shown in Fig. 3 (Type A and Type B). Type C grew so slowly and had so wrinkled surface that it was difficult to obtain the morphological data. The increase of frond area of juvenile sporophyte of D1+D2 and D2+D8 were shown in Fig. 4. Apparently, the big difference in the growth rate of the two plants investigated was genetically controlled, since all the young plants were cultured under the same conditions.

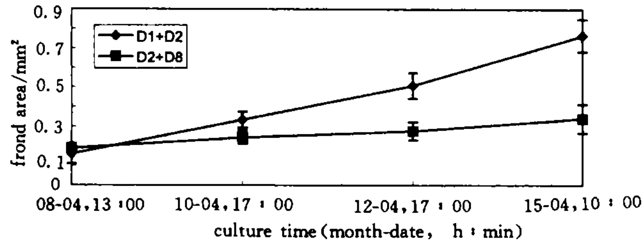

Fig. 4　Increase of frond area of juvenile sporophytes resulting from two corssings of *U. pinnatifida* (D1+D2 and D2+D8. For details, see materials and methods)

3　Discussions and conclusions

The success of multiplying vegetative gametophytes of *Undaria pinnatifida* by free-living techniques under a strictly controlled condition (Perez *et al*, 1984, Pang *et al*, 1996) makes seeding gametophytes possible for seedling production instead of seeding zoospores, and accelerates strain selection process which usually takes a few years for obtaining pure line of selfbred (Fang *et al*, 1983).

Adult sporophytes resulting from the same crossing combination of unicellular gametophytes possess identical morphology regardless of environmental factors (Pang *et al*, 1997). Results in this paper support this hypothesis for the early developmental stage of juvenile sporophytes. Vegetative gametophyte cells undergo multiplication through mitosis instead of meiosis, therefore, they are considered to be favourable materials for genetic study as illustrated by Fang *et al* (1983) and will be taken as an ideal materials for large scale seedling production in the coming years.

If the important economic characteristics of the studied algae, such as growth rate, higher pencentage of valuable contents of the plant, capacity against disease etc., can be determined by basic genetic analyses, and these characteristics can be passed stably to next generations, strain selection can be easily realized by crossing the parental unicellular gametophytes on large scale, thus greatly cutting down the time. In real production, some economic characteristics are considered more important than others. For example, the width of *U. pinnatifida* frond determines the percentage of the frond to total fresh weight, and therefore is an important characteristic.

Some questions still remain. The first is the lack of evidences that no substantial DNA changs occur in the process of million's time mitosis of vegetative gametophyte cells. Or specifically, whether genes controlling the expression of the characteristics change are not known yet. The second is based on the first one, ie, is it true that repeated use of the same crossing combination of unicellular gametophytes will not lead to the changes in the expression of related economic characteristics? The latter can be clarified by repeating the crossing experiment and open sea cultivation. This work is currently undertaken. For the first one, one needs molecular tools to find specific genetic markers. Unless some evidences against the observation made in the present paper occurs, it will continue to be a highlighting way for future strain selection program.

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裙带菜种内杂交研究
——幼孢子体形态和生长速度的变异 *

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提要　于 1995 年 3 月在日本 Tokushima 的裙带菜养殖基地分离和培养若干单倍体克隆并利用新近发展起来的单倍体丰富培养技术将分离的单倍体进行丰富培养。1996 年 3 月，以不同来源的裙带菜雌雄单倍体克隆进行不同组合的种内杂交实验，并利用多个单倍体克隆作为种源的对照。杂交所得到的不同幼孢子体在室内被培养在 15℃，L:D(光暗比) = 16:8，光强 255μmol / (m^2 · s)。在培养的过程中对幼孢子体进行了形态和生长的测定。生长的测定是利用计算机支持的生物图像自动测定系统(computer-aided bioimage system)完成的。这套系统可以准确的利用自动成像装置结合相应的软件来测定幼孢子体的面积的增长或连续的相对生长速度，这样可以克服只测量长度的增长而忽视宽度和整个面积的增长的缺点。结果表明：(1) 利用裙带菜单倍体克隆交配所得到幼孢子体(0.8—1.5cm)的形态具有高度的相似性，其生长速度也具有同步性；(2)不同单倍体克隆交配所得到幼孢子体的形态和生长速度具有显著的差异。因而，单倍体克隆交配所得到孢子体具有高度相同的形态特征在幼孢子体阶段得到证实。这一结果的意义在于可以通过筛选生长速度较快的杂交组合作为品系培育的基础材料，并结合具有其它的优良经济性状的单倍体克隆进行新品种的培育；由于使用单倍体克隆进行交配所得到的孢子体具有高度相同的性状这一工作假设在幼体和成体阶段均得到了证实，一个新的品系培育的方法更加趋于成熟，这为以后的育种工作奠定了理论基础。

关键词　裙带菜　种内杂交　配子体

学科分类号　Q32

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