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Genetic analyses of six quantitative traits of a doubled haploid population of *Porphyra haitanensis* Chang et Zheng (Bangiales, Rhodophyta)

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Abstract The first doubled haploid (DH) population of *Porphyra haitanensis* was created by crossing a wild-type line with a red type, artificial pigmentation, mutant line, by means of single somatic cell clone cultivation. Six quantitative traits (frond length (FL), frond width (FW), frond thickness (FT), fresh weight (W), growth rate of frond length (LGR), and growth rate of fresh weight (WGR)) from the DH population were analyzed. The frequency of each quantitative trait is in accordance with a normal distribution. Variable coefficients were between 20.43% and 57.35%, and while the mean of each quantitative trait was between the parents, it was closer to the paternal for six traits. Correlation analysis among the six traits showed significant positive correlations between FL and W, FW, and W, LGR and W, and WGR and W. There appears to be no correlation between FT and W. Heritability, number of genes controlling each quantitative trait and gene interactions, were also analyzed. Heritability of the six traits was greater than 70%, and heritability was not correlated with the number of genes controlling the corresponding quantitative trait. Frond thickness was the most heritable trait (95.30%), but this had the fewest control genes (7.52). According to the estimated coefficients of skewness and kurtosis, gene interaction was absent for LGR, but complementary gene interaction was observed in FW and W. In FL, FT, and WGR it is possible that complementary or duplicate gene interaction is involved. Our results enrich our understanding of *Porphyra* genetics and will help determine selection and breeding procedures for *P. haitanensis*.

Keywords *Porphyra haitanensis* · DH population · Genetic analysis · Quantitative traits · Selective breeding

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Introduction

Porphyra, a genus of marine red algae, is an important economic marine crop, with an annual harvest of more than 130,000 tons (dry weight) and a value of over US$ 2 billion (Sahoo et al. 2002). Farming and processing of *Porphyra* have generated the largest seaweed industries in East Asian countries; in China, Japan, South Korea, and North Korea (Sahoo et al. 2002). *Porphyra haitanensis* is one of the most important *Porphyra* species. It has been widely cultivated along the coasts of South China, especially in the Fujian and Zhejiang Provinces. In recent years, *P. haitanensis* has comprised 75% of the total production of cultivated *Porphyra* in China (Zhang et al. 2006). Although sea farming of *P. haitanensis* has been used since the early 1960s, most of the cultivated lines were wild varieties collected from the coast. These have been utilized over generations without selective breeding (Xie et al. 2009). Germplasm purification, rejuvenation, and genetic improvement have not been undertaken, resulting in the degeneration of the cultivar quality (Yan et al. 2010). It is, thus, highly desirable to select or breed new varieties of *P. haitanensis* that have strong economic traits and use these for cultivation.

Plant breeding is a dynamic area of applied science. It relies on genetic variation and uses selection to improve plant

characteristics that are of interest to grower and consumer. Many traits related to commercial production or quality of *P. haitanensis*, such as frond length, frond width, frond thickness, fresh weight, and growth rates, are quantitative characteristics (Miura 1976; Wu and Lin 1987). It has been believed that such complex traits are controlled by polygenes and will be susceptible to environmental change (James 2007). Usually, phenotypic variation in these traits depends on interaction between genes and environment, leading to a phenotype that has no specific corresponding genotype. This would serve to decrease the accuracy and efficiency of conventional breeding that depends directly on phenotype selection. Quantitative genetic methods allow us to determine to what degree phenotypic variation is genetically versus environmentally determined, and to understand the gene action for quantitative traits (James 2007). Following the results of quantitative genetic analysis, plant breeders and geneticists will be able to predict the response of each trait to selection and determine the best selection and breeding procedures. Quantitative genetic methods have been broadly used for terrestrial crop plants (Asins 2002). Among seaweeds with economic importance, Wang (1981) analyzed the heritability and genotypic correlation of some economic traits of *Laminaria japonica*. Liu et al. (2010) further analyzed the genetic bases of two quantitative traits (frond length and frond width) of *L. japonica* and located their genetic loci on a high-density map. However, genetic analyses of the quantitative traits of *Porphyra* have seldom been reported.

Doubled haploid (DH) populations are very useful in plant breeding. They can advance segregated generations of breeding material to homozygosity thereby reducing the time necessary to produce a new variety and increasing the efficiency of selection (Forster and Thomas 2005). Furthermore, DH lines can be powerful tools for genetic analysis, particularly of quantitative characters, since they form an immediate F_∞ generation with the advantages that this can provide (Snape et al. 1984). Doubled haploid populations of many plants have been constructed by microspore culture to analyze the genetic bases of complex quantitative traits and select new varieties.

A DH population of *P. haitanensis*, however, is not easy to construct due to the nature of its life cycle. Meiosis in *P. haitanensis* occurs during the first two divisions of the germinating conchospore. The initial four cells of a developing conchosporeling constitute a linear genetic tetrad, leading to the formation of chimeric blades of *P. haitanensis* (Yan et al. 2005, 2008). As they lack obvious markers, the sectors of a chimeric blade cannot be distinguished or separated to construct a doubled haploid. However, following the discovery of pigmentation mutants of *Porphyra*, different blade colors were used as genetic markers, thus distinguishing each sector of a chimeric blade, and enabling DH populations to be created (Xie et al. 2010).

In our study, the first DH population of *P. haitanensis* was created by crossing a wild-type line with a red type, artificial pigmentation, mutant line, by means of single somatic cell clone cultivation. Our primary goals were to analyze the variation in some quantitative traits relevant to commercial production or quality of *P. haitanensis*, based on our DH population and their parents, and to estimate heritability, the number of control genes, gene interaction, and the genetic correlations between quantitative traits. We hope that our results will enrich understanding of *Porphyra* genetics and help determine selection and breeding procedures.

Materials and methods

Construction of a DH population

The parental lines used were a wild-type line (male of *P. haitanensis*), YSIII, and a red type, artificial pigmentation, mutant line (female of *P. haitanensis*), RTPM. The free-living conchocelis of the wild-type line were established in 1999 from a gametophytic blade collected on the coast of Dongshan Island, Fujian Province, China, and maintained in the laboratory. The stock culture was maintained at 21±1°C, under 50–60 μmol photons m^{-2} s^{-1} [12:12 light/dark (L/D)] provided by cool, white, fluorescent lamps irradiance levels were check every 7 days. The culture medium (MES; Wang et al. 1986) was renewed every 6 months. Free-living conchocelis of the red-type artificial pigmentation mutant line of *P. haitanensis* were obtained by treatment of the gametophytic blades of another wild type with ^{60}Co-γ rays. This wildtype line was collected on the coast of Pintan Island, Fujian Province of china (Chen et al. 2008).

The mature free-living conchocelis of each parent were induced to release conchospores. These were collected in a 300 mL flask containing 200 mL of culture medium, and were cultured with aeration in an incubator at 25±1°C, under 80 μmol photons m^{-2} s^{-1} (10:14 L/D). Culture medium was renewed every week. Gametophytic blades developed, and after approximately 2 months in culture, healthy blades were selected as parents for crossing experiments. A male and a female blade were co-cultured in a flask until carposporangia appeared. About 2 weeks later, the fertilized female blade was transferred into a new flask and cultured under the same conditions until carpospores were released. The carpospores were collected and individually grown into conchocelis colonies in a test tube. When the colonies grew to 0.5 cm in diameter, they were fragmented by a homogenizer and continued in culture until conchospores were released. Culture conditions and methods were as described above. Once conchospores were released from the heterozygous conchocelis filaments, they were collected and passed gently through a 50-μm nylon

mesh filter, and cultured in Petri dishes containing the culture medium at 25±1°C, under 40 μmol photons m^{-2} s^{-1} (10:14 L/D). F1 gametophytic blades were obtained. After 40 days in culture, blades were picked out, transferred onto a glass slide, and examined under a light microscope (Nikon SMZ800). Samples from these partial color phenotype F1 blades were obtained by a puncher and each was digested into separated vegetative cells by 2% snail enzymes dissolved in 2 mol/L glucose liquor. The vegetative cells were then induced to develop into conchocelis (with double the normal number of chromosomes) by means of single somatic cell clone cultivation (Zeng et al. 2004), thus producing a DH population. Vegetative cells were induced to develop into the conchocelis stage using the following method. Single vegetative cells were isolated using a glass capillary and each cultured in one well of a 96-well plate. These stock cultures were maintained at 21±1°C, under 50–60 μmol photons m^{-2} s^{-1} [12:12 light:dark (L:D)] provided by cool, white, fluorescent lamps. The culture medium (MES; Wang et al. 1986) was renewed every 3 days. After culturing for 20-30 days, the majority of vegetative cells developed into conchocelis colonies. When the colonies wer 0.5 cm in diameter, they were fragmented using a homogenizer, culturing were continued in a 300mL flask under the same conditiond, until biomass sufficient. In our experiment, although 166 color sectors were gained from 50 F1 blades, only 157 color sectors developed into conchocelis.

Characterization of the gametophytic blades derived from each DH population and each parental line

Thirty healthy and integrated gametophytic blades derived from each DH population and each parental line were selected and put into three 1,000 mL flasks, with ten blades per flask. These were cultured with aeration in an incubator at 21±1°C, under 50–60 μmol photons $m^{-2}s^{-1}$ (12:12 L/D). The lengths of all selected gametophyte blades were 4.0±0.2 cm, and each flask contained 700 mL of medium, renewed every 2 days. The frond length (FL), width (FW), thickness (FT) and fresh weight (W) of the gametophytic blades were measured after 10 days in culture. The growth rates of frond length and fresh weight were calculated by the formulae:

$$\text{Growth rates of frond length (LGR)} = (\ln L_n - \ln L_0)/n \times 100\%$$

$$\text{Growth rates of fresh weight (WGR)} = (\ln W_n - \ln W_0)/n \times 100\%$$

where, L_n is the frond length of gametophytic blades that have been cultured for n days (cm), L_0 is the initial length of gametophytic blades, W_n is the fresh weight of gametophytic blades that have been cultured for n days (mg), W_0 is the initial fresh weight of gametophytic blades.

Statistical methods

The additive variance (V_a) components for each quantitative trait were estimated using the SPSS 13.5 analysis system. As our experiment used DH populations and each DH line was genetically homozygous at all loci, its genetic variance was equal to the additive variance (Caranta and Palloix 1996). Consequently, the broad sense heritability of the DH population was equal to the narrow sense heritability, so the heritability (h^2) of each quantitative trait was estimated as:

$$h^2 = V_a/(V_a + V_e)$$

where, V_e is environmental variance and is estimated by the average variance of each quantitative trait within 157 DH lines, and (V_a+V_e) means the total variation. The number of genes (k) controlling each quantitative trait was estimated as:

$$k = (L - m)^2/V_a$$

where, L is extreme value of each quantitative trait and m is the population mean of each quantitative trait (Choo and Reinbergs 1982).

Table 1 Performance of characters in the DH population and its parents

Characters	Paternal	Maternal	DH population	Coefficient of variation (%)	Asymptotic significance of one-sample Kolmogorov–Smirnov test (P_{ks})
FL (cm)	23.19±3.70[a]	34.08±3.90[a]	25.37±11.55	45.57	0.159
FW (mm)	6.82±1.12[a]	2.20±0.25[a]	5.86±2.00	34.03	0.277
W (mg)	78.43±16.66[a]	39.99±7.22[a]	75.40±43.24	57.35	0.398
FT (μm)	33.38±1.96[a]	26.25±1.77[a]	32.78±6.89	20.43	0.758
LGR (%)	17.06±1.06[a]	20.17±0.94[a]	16.64±4.84	29.07	0.944
WGR (%)	34.86±2.74[a]	24.20±2.00[a]	30.31±7.02	23.16	0.057

Data are the mean ± SD, t tests were used to analyze differences between parents, [a] highly significant ($p \leq 0.01$)

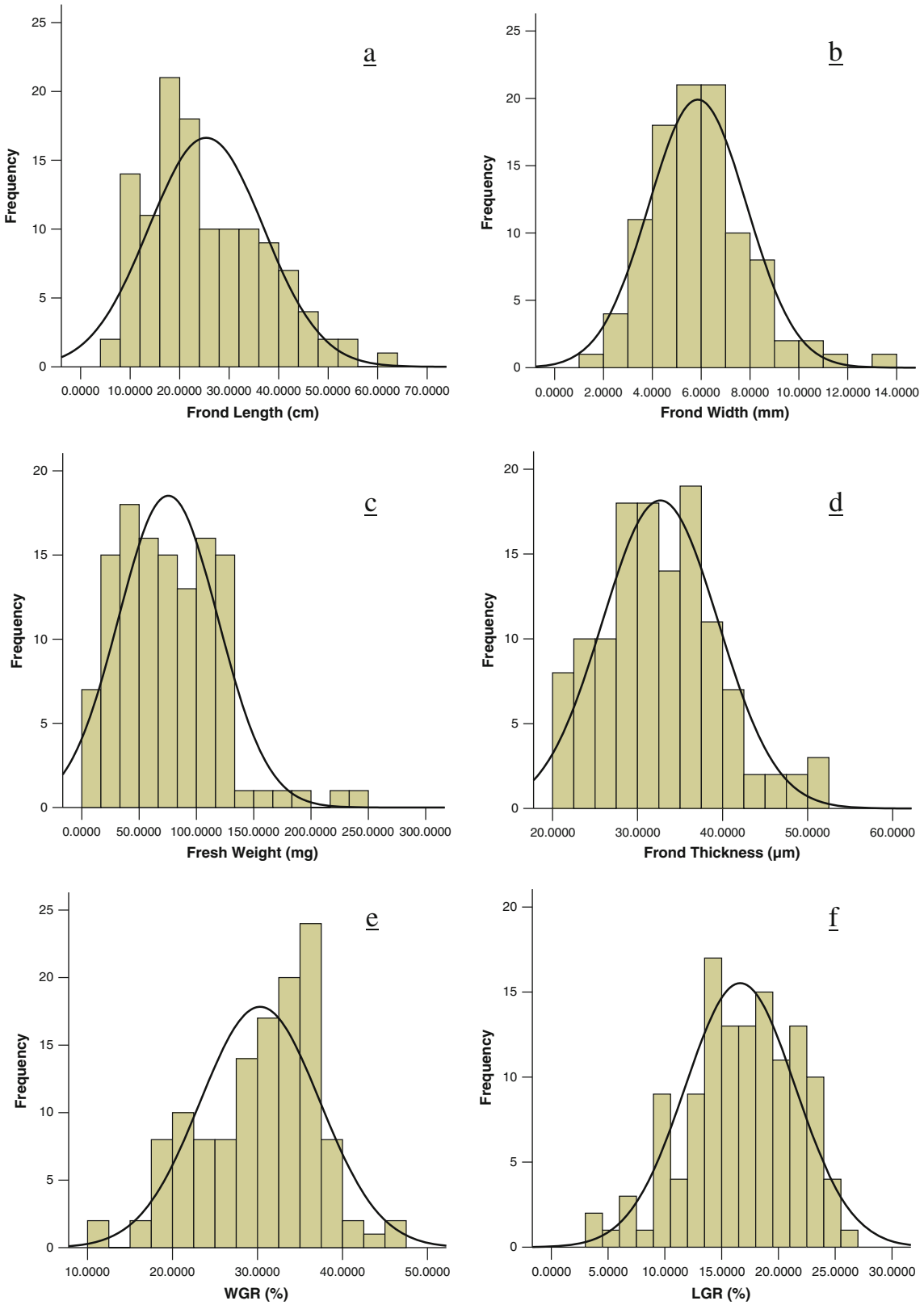

Fig. 1 Frequency distribution of each quantitative trait in the DH population, **a** fond length; **b** fond width; **c** fresh weight; **d** fond thickness; **e** growth rates of length; **f** growth rates of fresh weight

Table 2 Correlations between quantitative traits of *P. haitanensis*

	FL	FW	W	FT	LGR	WGR
FL						
FW	−0.116					
W	0.800[a]	0.422[a]				
FT	−0.241	0.195	−0.039			
LGR	0.950[a]	−0.096	0.774[a]	−0.144		
WGR	0.871[a]	0.034	0.781[a]	0.009	0.894[a]	

[a] Highly significant ($p \leq 0.01$)

In our estimation of gene effects and gene interaction, the coefficients of skewness (g_1) and kurtosis (g_2) were estimated for each quantitative trait, by the formula advanced by Choo and Reinbergs (1982). If the coefficients of g_1 and g_2 are not significantly different from zero, or if g_1 and g_2 are significantly smaller than zero, this indicates that gene interaction is absent. If the coefficients of g_1 and g_2 are significantly larger than zero, this indicates complementary gene interaction. If the coefficient of g_1 (g_2) is significantly larger than zero, and g_2 (g_1) is larger than zero but not significantly so, or if g_1 is significantly larger than zero, and g_2 is smaller than zero but not significantly so, this indicates the possibility of complementary gene interaction. If the coefficient of g_1 is significantly smaller than zero and *g2* is significantly larger than zero, this indicates duplicate gene action (Choo and Reinbergs 1982; Zhang et al. 2006).

Results

Analysis of quantitative traits from parents and DH populations

The wild-type line and red-type artificial pigmentation, mutant line of *P. haitanensis* (YSIII and RTPM) were used as parents. The paternal (YSIII) line exhibits slower growth, wider and thicker fronds, and forms spermatangia more easily. The maternal (RTPM) line exhibits faster growth, and narrower and thinner fronds. The 6 traits analyzed were: FL, FW, FT, W, LGR, and WGR. There were significant differences in each trait between the parents ($P<0.01$; Table 1). The maternal line was lower than the paternal for four traits (FW, W, FT, and WGR), but higher for two traits (FL and LGR).

Gametophytic blades characters from 157 lines of the DH population were analyzed (Fig. 1). We observed that all the traits were continuously segregated. The means of these 6 traits were between their parents, but in each case they were nearer to the paternal (Table 1). The coefficient of variation for the six traits was between 20.43% and 57.35%. The asymptotic significance of a one-sample Kolmogorov–Smirnov test showed that the frequency of the six traits in the DH population was in accordance with a normal distribution ($P_{ks}>0.05$; Fig. 1, Table 1). Absolute values of skewness (g_1) were less than 1.0 and those for kurtosis (g_2) were less than 2.0 (Table 3). All our results indicate that these six traits in the DH population are a result of polygenic inheritance.

The data gained from 157 lines of our DH population was analyzed (Table 2). Negative correlations were observed between FL and FW, FL and FT, FW and LGR, and W and FT, with correlation coefficients of −0.116, −0.241, −0.096, and −0.039, respectively. These were not statistically significant, however. In contrast, significant positive correlations were observed between FL and W, FL and LGR, FL and WGR, FW and W, W and LGR, W and WGR, and LGR and WGR, with correlation coefficients between 0.422 and

Table 3 Heritability, estimated number of genes controlling quantitative traits, and coefficients of skewness and kurtosis

Characters	Additive variation (V_a)	Total variation (V_a+V_e)	Heritability (%)	Means (μ)	Extreme value (L_1)	Number of genes (k)	Coefficient of skewness (g_1)	Coefficient of kurtosis (g_2)
FL	130.85	158.63	82.49	26.49	60.11	8.64	0.592[a]	−0.286
FW	4.21	5.62	74.90	5.86	1.38	14.87	0.865[a]	2.045[a]
W	1868.26	2465.56	75.77	72.41	237.55	14.07	0.883[a]	1.327[a]
FT	50.41	52.89	95.30	32.78	52.25	7.52	0.576[a]	0.114
LGR	20.66	24.22	85.31	16.64	3.57	8.27	−0.383	−0.289
WGR	41.95	51.91	80.81	30.31	10.02	9.81	−0.517[a]	−0.138

[a] Highly significant ($p \leq 0.01$)

0.950. Correlations between FW and FT, FW and WGR, and FT and WGR were also positive, but not significant.

Heritability, number of genes controlling each quantitative trait and gene interaction for six traits

The heritability (h^2) of each quantitative trait was estimated within the DH populations (Table 3). We observed that heritability of all six quantitative traits was greater than 70%. FT was the most heritable trait (95.30%), followed by LGR (85.31%), FL (82.49%), WGR (80.81%), W (75.77%), and FW (74.90%). Our results indicate that all six quantitative traits are greatly influenced by genetic factors, and can be reliably selected based on phenotype.

The number of genes controlling each quantitative trait was estimated for the DH population (Table 3). Our results indicate that the number of genes controlling FW is highest (14.87), followed by W (14.07), WGR (9.81), FL (8.64), LGR (8.27), and that FT has the fewest genes involved (only 7.52).

From the above data, we were able to show that there was a negative correlation between heritability and the number of genes controlling each quantitative trait. For one trait, for example, heritability was large and the number of control genes was small. Thus, the regression equation between heritability (X) and number of control genes (Y) can be deduced; the equation ($Y=e^{-0.748+251.183/x}$) and its curve are shown in Fig. 2. In this equation, the adjusted R square was 0.844, and the regression coefficient was significantly different from zero, consequently the equation can adequately explain the correlation between heritability and the number of control genes.

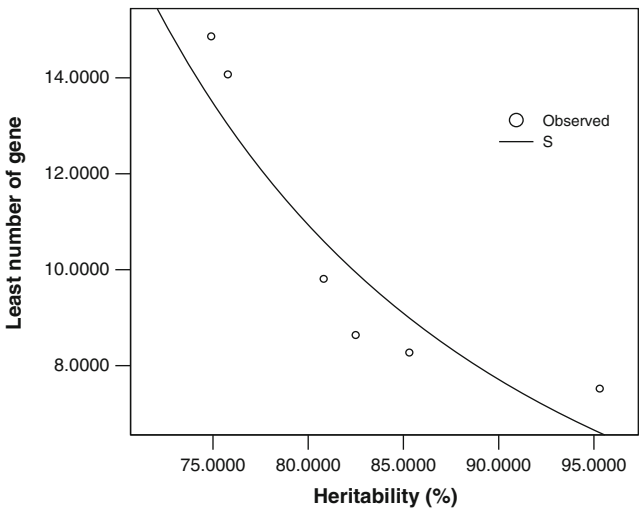

Fig. 2 The curve between heritability and the number of genes controlling each quantitative trait. The equation of the curve is $Y=e^{-0.748+251.183/x}$, ($R^2=0.844$, $p<0.01$)

We also tested the coefficients of skewness (g_1) and kurtosis (g_2) for each quantitative trait. Based on the g_1 and g_2 data, gene interaction was considered unlikely for LGR. However, complementary gene interaction was observed for the traits FW and W. The traits FL and FT also may exhibit interaction of complementary genes. The trait WGR may involve the interaction of gene duplicate genes.

Discussion

Variation within quantitative traits of *P. haitanensis* and their correlations

In plants of economic importance, quantitative variation is exhibited by many important traits, such as yield, quality or disease resistance (Asins 2002). In *P. haitanensis*, the characters FL, FW, FT, W, WGR, and LGR have implications for yield and quality, and exhibit continuous variation, showing a normal distribution in the DH population we studied (Fig. 1). This pattern validates the quantitative nature of these traits and hence their suitability for quantitative genetic analysis.

The coefficient of variation is an important index of the degree to which each quantitative trait varies. A larger variable coefficient indicates a higher probability of selecting a good variety from the population. A small variable coefficient could hamper the possibility of genetic enhancement from selective breeding. In our study, variable coefficients for the six traits were between 20.43% and 57.35%, and the variable coefficients of W, FL, and FW were higher than for the other three traits (Table 1). This implies that the former three traits are easy to improve by selective breeding. The lowest variable coefficient was for FT, and this indicates that FT has little potential for selective breeding. Similar results have been reported by Wang (1986) for *L. japonica*. Consequently, in designing a selective breeding program for a DH population, breeders should put most of their energy into selecting varieties of *P. haitanensis* that have longer and wider fronds, and high yield. However, FT is an important index of flavor for commercial dried blades in the nori industry (Yan and Aruga 2000). Thus, the breeder should also attach importance to selecting thinner fronds in *P. haitanensis*.

High yield, good quality, and faster growth are the three major targets in *Porphyra* breeding. These targets are correlated with several factors. Understanding these correlations is a prerequisite for breeding. As explained above, FL, FW, and FT are the factors influencing yield, and FT influences quality. Thus, these traits are regarded as the vital morphological features in the selective breeding of seaweed. WGR and LGR are two indices generally used to describe the growth rate of *Porphyra*. Table 2 showed that

the correlations between FL and W, FW and W, LGR and W, and WGR and W are significantly positive, but that there is no correlation between FT and W. To select high yield, good quality, and fast growing varieties of *P. haitanensis*, the breeder need only to select varieties with longer, wider, and thinner fronds.

Estimation of genetic parameters in the DH population of *P. haitanensis*

Methods of analyzing quantitative variation, and, in particular, of uncovering its likely genetic bases are therefore of prime importance in selective breeding. Heritability, number of genes controlling each quantitative trait, and gene interaction has been considered important parameters for quantitative genetic analysis by many researchers (Asins 2002). Our study provides the first estimate of heritability for several quantitative traits in *Porphyra*.

Heritability is the proportion of a population's phenotypic variation that is attributable to genetic variation among individuals. Analyses of heritability can allow us to estimate the relative contributions of genetic and non-genetic factors to the total phenotypic variation in a population, and the reliability of selection based on phenotype (Peter et al. 2008). In our DH population, heritability of all six quantitative traits was greater than 70%, and FT was the most heritable trait (95.3%). This indicates that all six quantitative traits were greatly influenced by genetic factors, and can be reliably selected based on phenotype. A similar investigation was conducted for *L. japonica* (Wang 1981), but results showed that FW was the most heritable (74.47%), and FT the least heritable of these traits (37.24%). Differences in heritability between these seaweeds are to be expected, because heritability estimates for a given trait are known to vary between different species or between different populations of a single species (Falconer 1989).

Estimating the coefficients of skewness (g_1) and kurtosis (g_2) for each quantitative trait can also help to determine the best selective breeding procedure. Following the theory of Choo and Reinbergs (1982), when gene interaction is observed, the breeder should reduce the selective power of any trait for which duplicate gene interaction has been observed to allow control genes to recombine. In contrast, traits that do not exhibit gene interaction, and have high heritability, should be selected for early in the breeding program. In the case of *P. haitanensis*, lines with longer, wider, and thinner fronds, should be selected for early in the breeding program due to the high heritability of two of these features.

Genetic analysis of the quantitative traits of seaweed is little reported in the literature. Our current methods of analyzing quantitative variation within seaweed populations should be improved, based on morphological characteristics. Our study enriches understanding of *Porphyra* genetics and helps to determine the best selection and breeding procedures for *P. haitanensis*.

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