BMC Genomics

RESEARCH

Open Access

Mechanisms underlining Kelp (*Saccharina japonica*) adaptation to relative high seawater temperature

Li Guo[1], Xiaojie Li[2], Shuxiu Chen[3], Yan Li[2], Weiwei Wang[3], Shiju Luo[2], Liming Jiang[2,4], Hang Liu[1], Xiaohui Pan[1], Yanan Zong[1], Leili Feng[1], Fuli Liu[1,5], Linan Zhang[6], Guiqi Bi[7] and Guanpin Yang[1,5,8,9*]

Abstract

Saccharina japonica has been cultivated in China for almost a century. From Dalian to Fujian, the lowest and the highest seawater temperatures in the period of cultivation increased by 14℃ and 8℃, respectively. Its adaptation to elevated seawater temperature is an example of securing the natural habitats of a species. To decipher the mechanisms underlining *S. japonica* adaptation to relative high seawater temperature, we assembled ~ 516.3 Mb female gametophyte genome and ~ 540.3 Mb of the male, respectively. The gametophytes isolated from southern China kelp cultivars acclimated to the relative high seawater temperature by transforming amino acids, glycosylating protein, maintaining osmotic pressure, intensifying the innate immune system, and exhausting energy and reduction power through the PEP-pyruvate-oxaloacetate node and the iodine cycle. They adapted to the relative high seawater temperature by transforming amino acids, changing sugar metabolism and intensifying innate immune system. The sex of *S. japonica* was determined by *HMG-sex*, and around this male gametophyte determiner the stress tolerant genes become linked to or associated with.

Keywords *Saccharina japonica*, High seawater temperature, Acclimation, Adaptation, Genome assembly, Genome wide association analysis, Transcriptomic analysis

*Correspondence:
Guanpin Yang
yguanpin@ouc.edu.cn
[1]College of Marine Life Sciences, Ocean University of China (OUC), Qingdao 266003, P. R. China
[2]Shandong Technology Innovation Center of Algae and Sea Cucumber, Shandong Oriental Ocean Sci-Tech Co., Ltd, Yantai 264003, Shandong, P. R. China
[3]Provincial Key Laboratory of Marine Seed Industry of Shandong, Shandong Oriental Ocean Sci-Tech Co., Ltd, Yantai 264003, Shandong, P. R. China
[4]Yantai Marine Economic Research Institute, Yantai 264006, Shandong, P. R. China
[5]Key Laboratory of Marine Genetics and Breeding of Ministry of Education, OUC, Qingdao 266003, P. R. China
[6]School of Marine Science and Engineering, Qingdao Agriculture University, Qingdao 266109, P. R. China
[7]Key Laboratory of Synthetic Biology of Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, P. R. China
[8]Provincial Key Laboratory of Marine Seed Industry of Shandong, College of Marine Life Sciences, Ocean University of China, Qingdao 266003, P. R. China
[9]Institutes of Evolution and Marine Bioaffiliationersity, OUC, Qingdao 266003, P. R. China

Introduction

The most intensively cultivated kelp species in China is *Saccharina japonica* [1, 2]. *S. japonica* is native to the cold-temperature coasts along northern Japan, northwestern Korea and far eastern Russia [3]. It was introduced into China from Japan and cultivated in northern China (Dalian, Shandong Peninsula) first and then in southern China (Fujian) [4, 5]. The genetic improvement and cultivation of *S. japonica* are based on our understanding of its life history, and our finding that both female and male gametophytes are isolatable and artificially culturable (cloneable) (Fig. 1A). The seedlings of *S. japonica* are raised indoor in precooled seawater with either gametophytes or sporophytes as parents [6]. The wild *S. japonica* sporophytes have been frequently documented on the cultivation facilities and the adjacent regions in northern China; however, their fronds are small and their distribution range is narrow [7]. The cultivated *S. japonica* has been used as human foods, animal feeds and industrial raw materials, and contributed also to the oceanic carbon sequestration [8, 9]. *S. japonica* immigrated > 3,400 km in distance and about 17 degree in latitude from Hokkaido (Japan) to Dalian, then Shandong Peninsula and Fujian coasts. From Dalian to Fujian, the summer seawater temperature increases from ~ 3℃ in Jan., < 1℃ in Feb. and ~ 17℃ in Jun [10]. to ~ 21℃ in Nov., 15℃ in Mar. and 25℃ in Jun. (Saojun Pang, personal communication). The lowest temperature increased by 14℃ while the highest temperature increased by 8℃. *S. japonica* has adapted to relative high seawater temperature. Brown algae may shift their habitats northward, and lose their diversity [11]. Unfortunately, the physical and genetic bases underlining the adaptation of kelp to relative high seawater temperature remain unclear.

Genetically, the sex is a separating and mixing process of genomes, which encompasses diverse systems and mechanisms [12]. Except for the sex chromosomes and master sex determination gene, intrinsic and extrinsic environments may also play roles in sex determination. The model brown alga *Ectocarpus* determines its gender with the sex determination region on U (female) and V (male) chromosomes, respectively [13, 14], and the *HMG-sex* has been identified as the male-determiner of *Ectocarpus* gametophytes [15]. When U and V chromosomes reunite in diploid sporophytes, they are extensively regulated and no longer determines the sex [16]. The response of organisms to environmental change can depend on the biological gender while the sex-basis induced by the environmental change may cause the habitat shifting and the loss of biodiversity [17]. The density of vertebrates with temperature-dependent sex determination may collapse under the extreme thermal fluctuations [18]. High temperature may lead to the overproduction of one sex and, in turn, biases the sex ratio of populations [19]. In comparison, the location, the gene components and the structure of *Saccharina japonica* sex determination regions have not been characterized. In addition, kelp breeders have been dedicating to develop high temperature tolerant varieties; such varieties may extend growing time and increase yield and harvesting and post-harvesting periods. However, the studies on the differential responses to relative high seawater temperature between kelp gametophyte genders are scarce.

With the gametophytes isolated from the kelp cultivars of northern and southern cultivation regions of China, the acclimation and adaption mechanisms of kelp to relative high seawater temperature were addressed in this study.

Materials and methods

Isolation and biomass amplification of Kelp gametophytes

The gametophytes were isolated from mature kelp sporophytes with the method we described early [6, 10]. The gametophyte clones were maintained at Kelp Species and Elite Variety Center, Shandong Oriental Ocean Sci-tech, Yantai, Shandong, China. The biomass of gametophyte clones was amplified with the method we described early [10]. The gender of the gametophyte clones was verified with sex-specific microsatellite alleles and sex-unique morphological characteristics as we did early [20, 21].

Contig assembling

Gametophyte clones, female and male each, ~ 100 mg in wet weight, were collected separately through brief centrifugation, ground into fine powders in liquid nitrogen, and transferred into a pre-chilled centrifugation tube. Genomic DNA was isolated with CTAB method.

The whole genomes of *S. japonica* male and female gametophytes were sequenced separately with the SMRT sequencing technology. The high-quality reads were assembled into contigs using falcon (v0.2.0), and the errors in the primarily assembled contigs were identified and corrected with the arrow tool. The genomic DNA was used also to construct the short fragment library which was then sequenced on Illumina platform, and used to correct the errors in contigs. The short fragment libraries were also constructed and used to re-sequence the gametophyte populations, identify the sex determination region of kelp gametophytes and decipher the genetic basis associating with the tolerance to relative high seawater temperature.

Upgrading contigs into scaffolds

The biomass of the gametophyte clones, both male and female, was harvested separately and used to construct Hi-C library which was then sequenced on Illumina platform. The read pairs generated were mapped to the contigs. According to the mating and concomitance

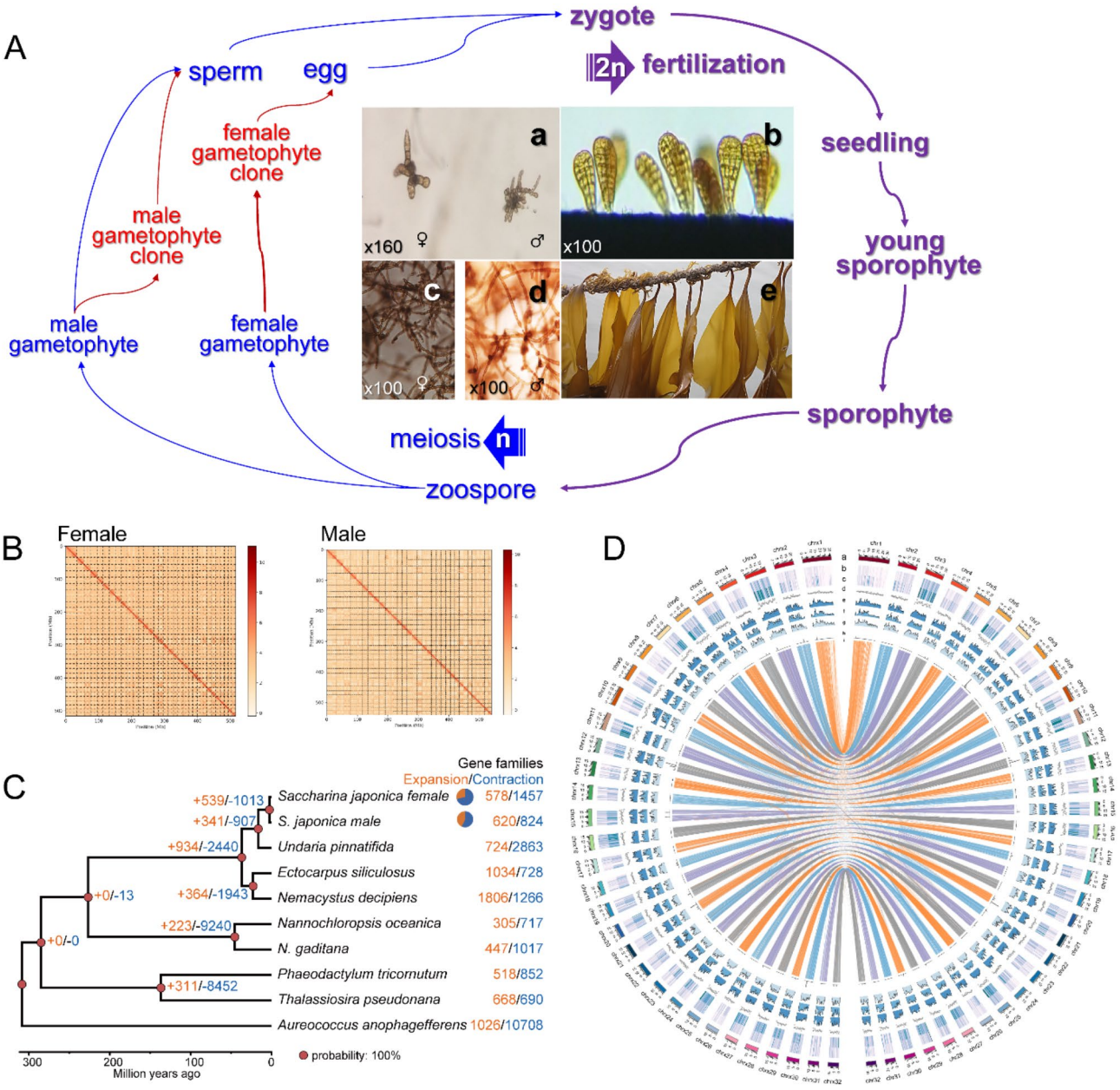

Fig. 1 The life cycle of *Saccharina japonica* (**A**) and graphical display of *Saccharina japonica* gametophyte genome assemblies (**B** - **D**). The gametophytes can be vegetatively cultured (cloned), and preserved as the genetic resources. The gametophyte clones can be hybridized with the first filial generation used as either the hybrid kelp or the basic population of selection breeding. **a**, the gametophytes germinated from zoospores; **b**, young sporophytes; **c** and **d**, artificially cultivated gametophytes (clones); and **e**, sporophytes under cultivation. **B**, the interaction map of the scaffolds revealed with the high-throughput chromosome conformation capture (HiC) technique. Thirty-two scaffolds showing the strongest interactions (the color bar indicates the interaction strength, the deeper the stronger) are selected as the skeleton ones which are synonymous with chromosomes throughout the context. **C**, the phylogenetic tree of *S. japonica* gametophyte genomes and those of the representative species in Stramenopiles (Heterokonts)-Alveolates-Rhizaria (SAR) group. The Bayes tree is constructed using 397 single copy genes shared among the representative species of SAR group. The genomes of *S. japonica* gametophytes are more similar with those of *Nannochloropsis* than with other representatives of SAR group. *Aureococcus* serves as the outgroup. The red dots at each node are the bootstrap value (100%) while the plus and minus numbers at each node are the gene families experienced expansion and contraction, respectively. The numbers in the right-hand column are the expansion and contraction gene families each species. Except the sex determination region of U and V sex chromosomes, the genomes of female and male gametophytes are almost identical. The seeable differential in the numbers of expansion/contraction gene families between female and male gametophyte genomes should result from the difference in the completeness and non-overlap area of the assemblies. **D**, the characteristics of *S. japonica* chromosomes. The tracks from outer to inside are chromosome length in megabase (**a**), density of genes annotated along forward strand in 500 kb non-overlapping windows (**b**), density of genes annotated along reverse strand in 500 kb non-overlapping windows (**c**), number of genes (**d**), the distribution of LTR (**e**), DNA transposons (**f**), LINE (**g**) and SINE (**h**), and the syntenic gene pairs identified between female (right semicircle) and male (left semicircle) gametophyte genomes (**i**)

(chromatin interaction) information, the contigs were preliminarily clustered, ordered and oriented, yielding skeleton, contaminated and unanchored scaffolds. The skeleton scaffolds were used as the genome assembly.

Gene prediction and annotation

The protein encoding genes were identified with three methods, ab initio modeling, homology-based predicting and transcriptome-aided predicting. To carry out transcriptome-aided prediction, total RNA was extracted with TransZol Plant Kit (Invitrogen). The mRNA was separated from the total using poly-T coated magnetic beads, fragmented, and used as the templates of the first-strand cDNA synthesis. The second-strand cDNA was synthesized subsequently. The double-stranded cDNA was sequenced. The clean RNA-seq reads of *S. japonica* gametophytes and sporophytes at different developmental stages were assembled into transcripts. The predicted genes were functionally annotated according to their best hits to the deposited in databases. The completeness of the assembly was evaluated using BUSCO against OrthoDB. Diverse repeats were identified according to sequence similarity. The genes encoding tRNAs were identified using rice tRNAs as queries. To identify rRNA genes, the orthologs of the closest species were downloaded and aligned with the assembly. The snoRNAs, MiRNAs, and snRNAs were identified against Rfam database. The repeats were either identified with RepeatMasker (open-4.0.9) and RepeatProteinMasker (open-4.0.9) based on RepBase database or predicted using LTR_FINDER (v1.0.5) and Repeat Modular (open-1.0.11) from scratch. The TRF was also used for the repeat annotation with TRF (v4.09). The identified repeats were integrated with the redundant ones eliminated.

Genome comparison and gene phylogenetic analysis

The amino acid sequences of the annotated genes of the genomes selected for genome comparison were clustered into orthogroups with Orthofinder, from them the gene trees of all orthogroups, the rooted species tree, and all gene duplication events were inferred. The rooted gene trees were generated with Orthofinder to infer the orthologs by phylogenetic information with corrected tree topology. For the phylogenetic analysis, the sequences were aligned with ClustalO. The phylogenetic tree was constructed using MrBayes.

Resequencing and GWAS

From the genetic resource library maintained at Kelp Species and Elite Variety Center, Shandong Oriental Ocean Sci-tech, Yantai, Shandong, China, 94 gametophyte clones, half male half female, were selected and amplified in biomass. These clones were isolated from the kelp sporophytes from different habitats and in different years, which should represent the north kelp cultivation sea area, Shandong and Liaoning Provinces, China. The gender of these gametophyte clones was re-verified with sex-specific microsatellite alleles and sex-unique morphological characteristics as we did early [20, 21]. To decipher the genetic basis underlining the warm seawater tolerance of kelp gametophytes, 36 gametophyte clones were established from the south cultivation sea area, Fujian Province, China, half female and half male, and maintained at Kelp Species and Elite Variety Center, Shandong Oriental Ocean Sci-tech, Yantai, Shandong, China. The biomass of these clones was amplified with the methods we described early [6, 10].

These gametophyte clones were resequenced with the methods described in contig assembling section. The clean reads were mapped to the female genome assembly obtained in this study with BWA. The duplicate reads were removed using SAMtools and Picard version 1.106. The high-quality paired reads were filtered with the threshold of Phred score ≥ 30 and the raw SNPs were called with the threshold of base quality Phred score > 20 with GATK. SNPs implemented in GATK were joined with HaplotypeCaller.

The raw SNPs were filtered with GATK following the standards including quality by depth < 2.0, Phred-scaled p value (FS) > 60.0, root mean square of the mapping quality < 40.0, variants with mapping quality rank-sum test approximation < – 12.5 and a read position rank-sum test approximation < – 8.0. The SNPs were further filtered with VCFtools with the threshold of genotype quality ≥ 30, the minimum mean depth of coverage ≥ 10, the maximum mean depth of coverage ≤ 50 and no missing data. Plink was used to convert the SNP data from VCF format into PED/RAW and MAP/BIM. Multispecies coalescent based analyses were performed against a dataset of 3,075 haploblocks of a minimum 25 kb in length containing minimum 50 biallelic sites with a minor allele frequency of 0.2 or higher. These haploblocks were detected using Plink with–blocks option by setting block-max-kb and blocks-min-maf to 1,500 and 0.2, respectively. Linkage disequilibrium estimates within and between haploblocks were visually inspected using LDBlockShow.

The associations between a SNP and gender was found by χ^2 testing the independence of the SNP in female (case) and male (control) populations consisting of the gametophyte clones from the north cultivation sea area of China. The SAIGE method was used to identify SNPs associating with the high seawater tolerance of gametophytes by treating 100 gametophytes representing the north cultivation sea area of China (case) and 30 gametophytes representing the south cultivation sea area of China (control), respectively.

RNA-seq

To document the physiological mechanism underlining the high sea water tolerance of kelp gametophytes, we selected 2 types of genotypes representing the north and south cultivation sea areas, respectively, 12 gametophytes each, half female and half male, which were treated at normal temperature (18 ℃) and relative high temperature (23 ℃) separately for 12 h, 12 gametophytes each temperature, 6 from south and 6 from north, half female and half male. In total, 24 transcriptomes were sequenced.

The transcriptomic analysis was carried out with the method described in gene prediction and annotation section. The clean reads were mapped to the female gametophyte genome assembly with HISAT2. The differently expressed genes (DEGs) were calculated with DESeq at the threshold of|log2FoldChange| ≥ 1 and FDR ≤ 0.05. The result of Gene Ontology (GO) enrichment was analyzed using goseq and visualized using REViGO. KEGG enrichment was analyzed using R and BH correction with q-value ≤ 0.05.

Table 1 The characteristics of Kelp (*Saccharina japonica*) female and male gametophyte genome assemblies

	Female	Male
No. of contigs	1,217	819
No. of N_{50} contigs	155	119
Length of N_{50} contigs (bp)	977,358	1,337,703
Maximum contig length (bp)	5,667,585	8,305,191
Total contig length (Mb)	515.7	594.2
No. of chromosomes	32	32
Maximum chromosome length (bp)	33,688,922	31,428,625
Total chromosome length (Mb)	516.3	540.3
No. of genes	15,010	15,849
Average length of genes (bp)	15,952	8,374
Average length of CDS (bp)	1,550	1,557
Average no. of exons	7.6	4.3
Average length of exon (bp)	205	203
Average length of intron (bp)	1,923	1,139
Supported by transcripts (%)	72.5	72.0
BUSCO score (%)	86.1	83.5
Total length of repeats (bp)	362,951,269	382,613,256
Percentage of repeats	70.3	61.8
Length of DNA transposon (bp)	48,993,216	57,433,098
LINE (bp)	73,873,712	76,921,424
SINE (bp)	1,266,119	1,492,227
LTR (bp)	248,067,874	264,212,052
Total length of tRNA gene (bp)	32,301	34,169
No. of tRNA gene	431	450
Total length of rRNA (bp)	5,793	8,109
No. of rRNA gene (bp)	5	9
Total length of snRNA (bp)	7,713	7,537
No. of snRNA gene	51	51

Results

Characteristics of *S. japonica* gametophyte genome assemblies

The genomes of a *S. japonica* female gametophyte and a male gametophyte were sequenced on the PacBio platform. The reads were assembled into contigs, and subsequently into chromosomes with the aid of positional information revealed with HiC method. Thirty-two skeleton scaffolds showing the strongest interactions as the color bar indicated (the deeper the stronger) were selected as the chromosomes of kelp gametophytes, both female and male (Fig. 1B). The total length of female chromosomes was ~516.3 Mb while that of male gametophyte was ~540.3 Mb. The length of female contig N_{50} reached 977,358 bp with the maximum of 5,667,585 bp while that of male contig N_{50} reached 1,337,703 bp with the maximum of 8,305,191 bp. The female and male assemblies contained 15,010 and 15,849 protein-encoding genes, of them 72.5% and 72.0% corresponded to the transcripts. A total of 70.29% female genome and 61.67% of male genome were occupied by repeat-sequences. The BUSCO evaluation showed that the female and male genomes encompassed 86.1% and 83.5% of the annotated protein homologs, respectively (Table 1). *S. japonica* belongs to the heterokonta of SAR group. The Bayes tree constructed using 397 single copy genes shared among the representative species of SAR group showed that *S. japonica* gametophyte genomes are more similar with those of *Nannochloropsis* than with other representatives of SAR group (Fig. 1C). Other characteristics of *S. japonica* chromosomes including the density of genes, number of genes, the distribution of repeat elements among others and the synteny of annotated genes between female and male gametophyte genomes were also documented and visualized (Fig. 1D).

Brown algae are a eukaryotic lineage that shares the innate immune system with animals, fungi, plants and green and red algae [22, 23]. The innate immune is also linked to the iodine metabolism [24]. The extrinsic pathogens may initiate the formation of elicitor oligoguluronate (GG) which may also be yielded by intrinsic alginate lyase [22, 25]. Among plants, the gene retention after polyploidization and the gene expansion in mono- and diploid genomes strengthen their stress tolerance [26, 27]. It is common that plants and brown algae respond to stress by generating reactive chemical species [22, 28]. We identified the genes encoding alginate lyase, phospholipase, respiratory burst oxidase homolog (RBOH), aquaporin, vanadium-dependent bromoperoxidase among others involved in kelp innate immunity, and found that their genes are highly repeated (expanded) with the copy number varied between 2 and 26 in the female gametophyte genome.

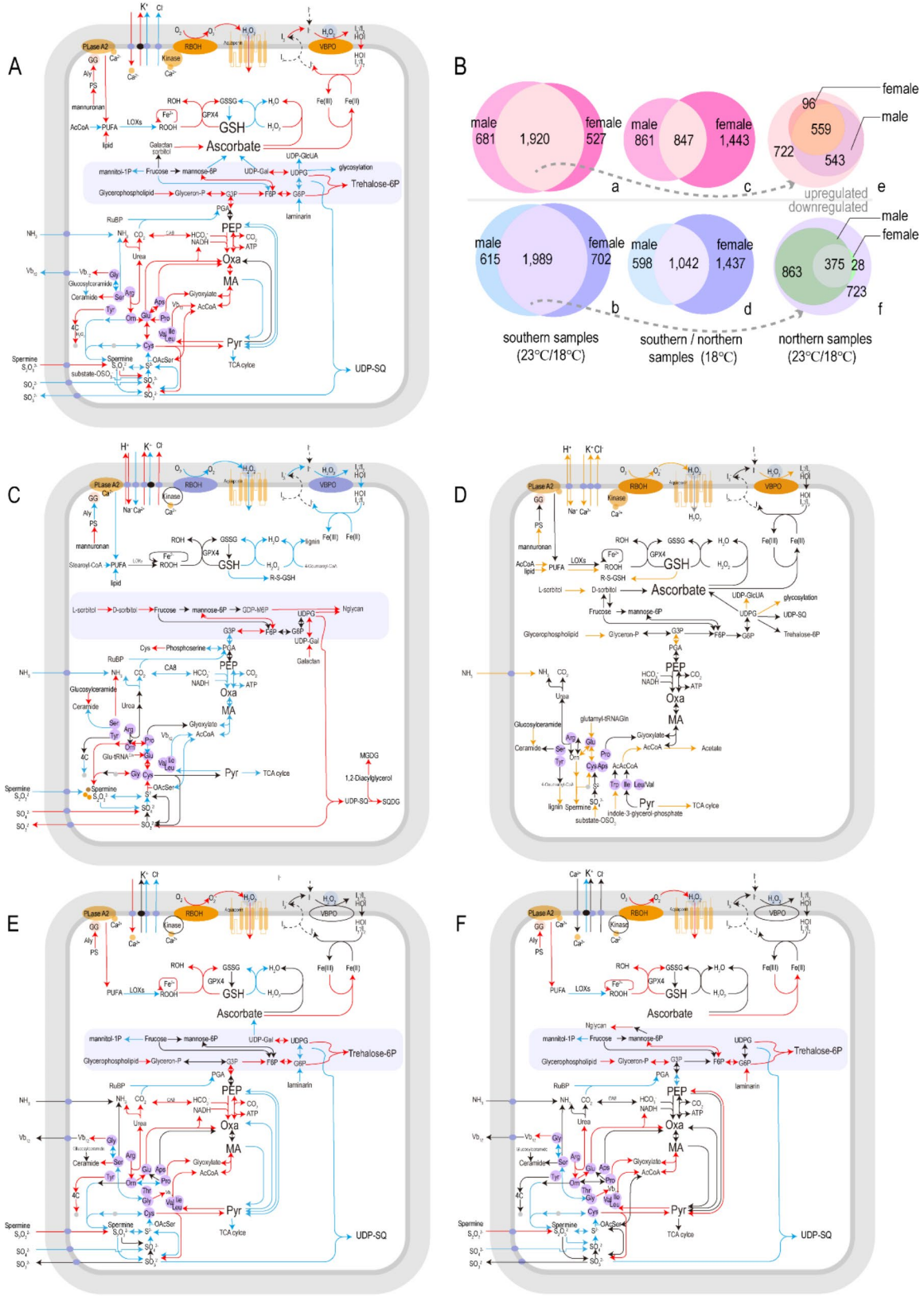

Fig. 2 (See legend on next page.)

(See figure on previous page.)

Fig. 2 Genetic and transcriptomic analyses of northern and southern gametophytes of *S. japonica*. **A**, the function-known genes identified through whole genome association analysis, which contain SNP(s) within them. **B**, the differentially expressed genes (DEGs) between southern and northern gametophytes (male vs. female. **a**, up-regulated; **b**, down-regulated); and when the southern gametophytes growing at 18℃ are shifted to 23℃ (male vs. female. **c**, up-regulated; **d**, down-regulated). Of the shared DEGs of southern gametophytes when shifted from 18℃ to 23℃, some are shared by southern gametophytes (**e**, up-regulated; **f**, down-regulated). **C**, The DEGs between southern and northern gametophytes identified when they are cultivated at 18℃ and the intensified and weakened metabolism routes involving these DEGs. The philosophy is followed when we trace the metabolic fluxes, *e.g.*, a metabolism route connecting two metabolites within and among KEGG pathways is believed to be intensified (red arrowed line) if all DEGs are up-regulated, weakened (blue arrowed line) if all DEGs are down-regulated, otherwise uncertain (black arrowed line). **D**, the DEGs and the metabolism routes when southern gametophytes are shifted from 18℃ to 23℃. **E** (male) and **F** (female), the DEGS and the metabolism routes when northern gametophytes are shifted from 18℃ to 23℃

Acclimation of southern gametophytes to relative high seawater temperature

We identified the differentially expressed genes (DEGs) according to the abundance of gene transcripts, and assigned these DEGs to the KEGG pathways. DEGs standing alone and the KEGG pathways were combined to trace the metabolism route between two metabolites of concern. A metabolism route is believed to be intensified if all DEGs along it are upregulated, and weakened if all DEGs are downregulated; otherwise, the route is not analyzed further. In addition, all samples in a group must show the same trend of regulation regardless of occurrence.

Following this principle, we found that the whole innate immune process was intensified when the southern gametophytes maintained at 18℃ were cultivated at 23℃ (Fig. 2ABa, b). The transcript abundance of the intrinsic alginate lyase gene increased, which degraded the alginate into GG dimer and then activated the phospholipase, yielding PUFA and strengthening the influx of calcium ion. The PUFA was then oxidized into hydroperoxide (ROOH) which can be reduced into alkanol (ROH) by glutathione (GSH). Simultaneously, the calcium ion activated the kinases and then RBOH, yielding apoplast hydrogen peroxide (H_2O_2). Hydrogen peroxide went into the cell *via* aquaporin, where it was reduced into water. It was interesting to notice that GSH remained almost constant when ROOH increased. To reimburse GSH demand, ascorbate was synthesized from carbohydrate, which eliminated H_2O_2 and transformed ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}). The ascorbate performance can be coupled with iodine cycle strengthening, exhausting the energy when kelp met high seawater temperature. Glutathione and ascorbate detoxify the reactive oxygen species (ROS), protecting cells from abiotic damages [29, 30].

At high seawater temperature, *S. japonica* strengthened its synthesis of glucose-6P, attenuated its synthesis of UDP-sulfoquinovose (UDP-SQ), and intensified its synthesis of trehalose from glucose-6P and uridine diphosphate glucose (UDPG), which aided to maintain its cellular osmotic pressure [31]. *S. japonica* regulated the expression of its diverse transporter and enzyme genes, transforming its sulfur groups and amino acids [32, 33]. Different organisms share twelve precursor metabolites, of them phosphoenolpyruvate (PEP), pyruvate and oxaloacetate comprise a node, the heart of metabolism [34, 35]. The transformed amino acids flew into the heart of metabolism; however, no outlet was found. Running this node consumes energy and reduction power. Ticking over this node assisted the alga to tolerate relative high seawater temperature. Plants defend against stresses and suppress growth simultaneously [36]. The southern kelp sporophytes cannot survive the summer seawater temperature, and cannot grow into fronds with normal size even they were cultivated in northern cultivation areas. This may explain why the wild kelp in small size and narrow distribution were often documented in northern kelp cultivation region.

Adaptation of southern gametophytes to relative high seawater temperature

The differential gene expression may be due to either fluctuation of gene transcription (acclimation) or genetically controlled variation (adaptation) [37, 38]. The DEGs between southern and northern gametophytes when they are cultivated at 18℃ and the metabolism routes defined by these DEGs indicated that *S. japonica* intensified its syntheses of Nglycan and sulphoquinovosyl diacylglycerol (SQDG) (Fig. 2CBc, d). SQDG and monogalactosyldiacylglycerol (MGDG) function in membrane structure maintenance, photosynthesis and adaptation to environmental stresses [39, 40], and asparagine-linked-glycans (Nglycans) modifies proteins and mediates cellular recognition and host-pathogen interactions [41, 42]. The southern gametophytes adapted relative high seawater temperature by expressing these DEGs and through the pathways these DESs defined.

Genetic changes supporting the adaptation of southern gametophytes to relative high seawater temperature

Genome-wide association analysis (GWAS) identified 6,920 genic SNPs significantly different between gametophyte populations from southern and northern cultivars, respectively, which defined 80 annotated genes expressed differentially between southern and northern gametophytes at 18℃, and among southern gametophytes when they were cultivated at 18℃ first and then at 23℃. It was found that these genes encoded the enzymes

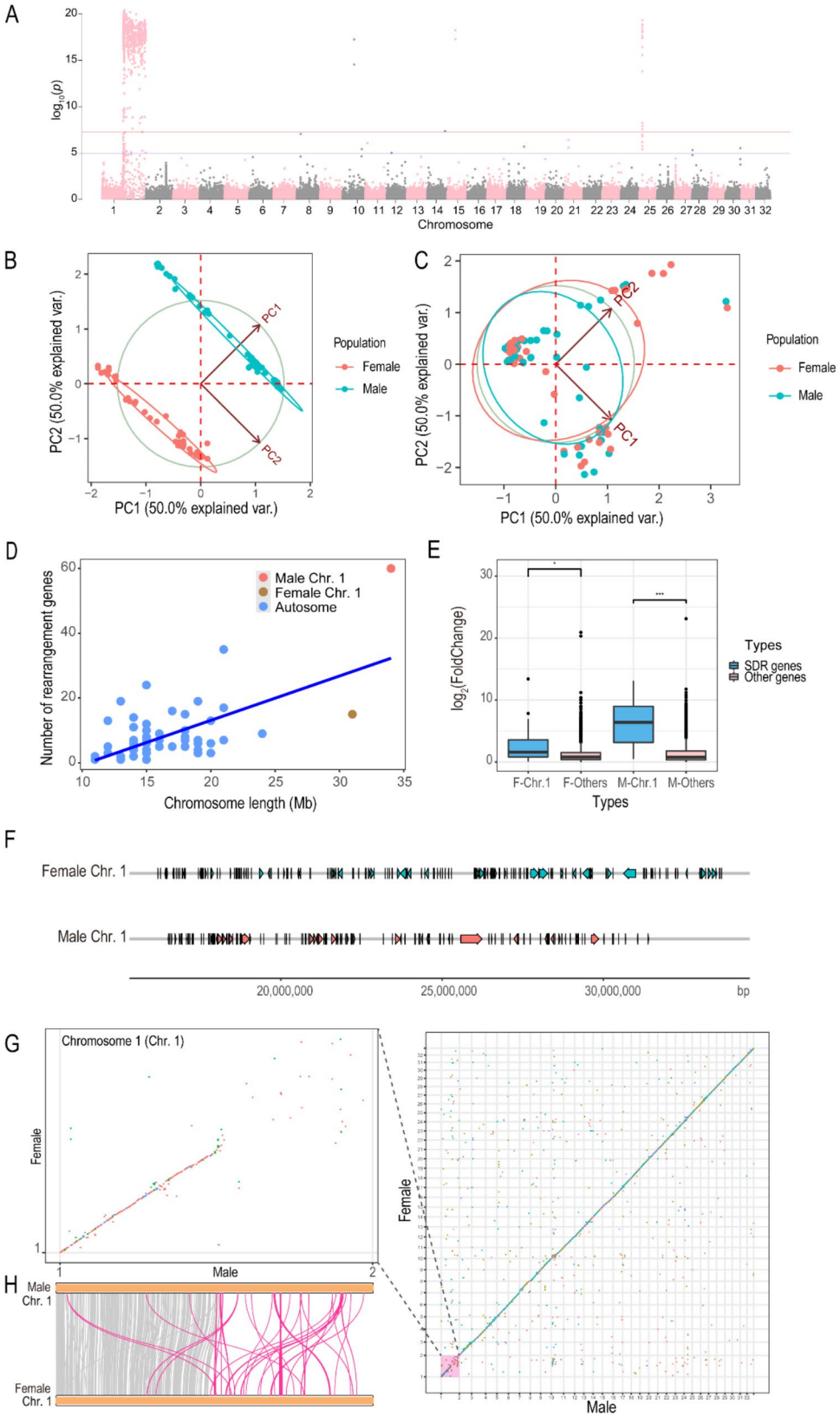

Fig. 3 (See legend on next page.)

(See figure on previous page.)

Fig. 3 Identification of sex chromosomes and establishment of the boundaries of sex determination region (SDR) within them. **A**, collinearity between male and female genomes. The red block shows the collinearity between male and female chromosome 1, which is enlarged to highlight the perfect collinearity of the first half and the non-existent collinearity of the second half of chromosome 1. **B**, collinearity of homologous genes located in male and female chromosome 1. The lines and dots in **A** and **B** represent the homologous genes, and the red lines and red dots in **A** and **B** refer to the genes not completely colinear while the gray lines and dots refer to the genes completely colinear. **C**, Manhattan plot of significantly different SNPs along 32 chromosomes of *S. japonica*, which are identified thorough GWAS. Accordingly, chromosome 1 is identified as the sex chromosome, and the second half of chromosome 1 is identified as the SDR. The chromosome 1 of female gametophytes is U chromosome while that of the male is V chromosome. The red line indicates the possibility of $5 \times 10-8$ while the blue one indicates the possibility of 10^{-5}. **D**, principal component analysis (PCA) of the SNPs in the first half (0-18 Mb) of chromosome 1. **E**, PCA of the SNPs in SDR. **F**, gene distribution of SDR in U and V chromosomes. The color arrows represent genes predicted, which shrink into vertical bars when the genes are small. **G**, the statistics of rearranged genes in U and V sex chromosomes and autosomes. **H**, gene expression of male- or female-biased genes in SDR and other regions

functioning mainly in amino acid transformation and innate immune process (Fig. 2D), which roughly corresponded to the metabolism processes defined by DEGs between southern and northern gametophytes when they were cultivated at 18℃ (Fig. 2C). Such correspondence indicated that these genes were genetically changed, and these metabolism routes were adaptive. Salt and thali tolerances of crops are more complex than anti-freezing [43–45]. The heat or high temperature tolerance is also a complex trait. Temperature determines the species distribution on earth even [46, 47]. The evolution of kelp tolerance to relative high seawater temperature is slow and gradual.

Differential adaptation of southern gametophytes to relative high seawater temperature between genders

A brief temperature increase profoundly influences spermatogenesis, but not oogenesis; such increase activates transposons, and provide a time window for them to mobilize and modify genomic DNA without affecting reproduction [48]. Spermatogenesis is more sensitive to small temperature increase; such increase multiplies the double-strand DNA breaks (DSBs), and concurrently reduces male fertility [49]. Brown algae have imitated animals in evolution in determining their male gender [15]. The transcriptomic analysis showed that northern male gametophytes upregulated their PEP-pyruvate-oxaloacetate node, exhausting more energy and reduction power by ticking over this node (Fig. 2E and FBe, f). The male gametophytes are more tolerant to relative high seawater temperature than the female.

Identification of sex chromosome and establishment of SDR boundaries

Genome-wide association studies (GWAS) identifies genetic marker across genomes or the genes associate with or determine a trait [50]. The sex determination region (SDR) within the U and V chromosomes of *S. japonica* was identified as a region from 16,179,947 to 33,672,368 bp of chromosome 1 (Fig. 3A), within which, 1,532 SNPs were found to be significantly different in frequency between male and female gametophytes. The gametophytes can be partitioned into two populations through PCA of SNPs in this region (Fig. 3B)but not those out of this regions (Fig. 3C). The evidences seconding this identification included also the higher rate of gene rearrangement in sex chromosomes than in autosomes, and in V chromosome than in U chromosome (Fig. 3D), and the higher number of sex-biased gene transcripts in sex chromosomes than in autosomes, and in V chromosome than in U chromosome (Fig. 3E). The SDR of V chromosome contained 186 genes while that U chromosome contained 134 genes (Fig. 3F). Most of the genes arranged linearly; however, some did not, and some located out of the SDR even (Fig. 3G, H).

Sex determination and SDR evolution of *S. japonica*

Morphologically identical autosomes may evolve into heteromorphic sex chromosomes which terminate recombination of sex-linked genes and sex-beneficial alleles, and vertically transfer these genes and alleles as a unit [12]. A sex-determination gene with unknown origin initiates the evolution of sex chromosomes [51, 52]. In the organisms with haploid-phase sex determination systems, the sex is determined genetically by U and V sex chromosomes with sex-determining region (SDR) each [53, 54]. The *Ectocarpus* U and V chromosomes have distinct SDRs, similar in size, structure and gene number (about 20), but different in gene types and gene expression pattern [14]. As was expected [55], the *HMG-sex* located on the SDR of V chromosome has been identified as the master male-determiner in *Ectocarpus* [15].

In the SDR of V chromosome of *S. japonica* male gametophyte, the homolog of *Ectocarpus HMG-sex* was identified, indicating that the origin of kelp sex determination was similar with that of *Ectocarpus*. However, GWAS showed that the genes have been differentially aggregating in the SDR of V or U chromosomes. Some genes were found to locate out of the SDR of V and U chromosomes (Fig. 3H) and in autosomes even (Fig. 3A). This finding implied that the kelp SDR is evolving.

Among the sex-associated genes, the *Ulp1* (ubiquitin-like protein specific protease, Ulp) was found to locate at autosome 14. Sumoylation modifies proteins with the small ubiquitin-like modifier protein (SUMO). The proteases in Ulp1 family control SUMO function

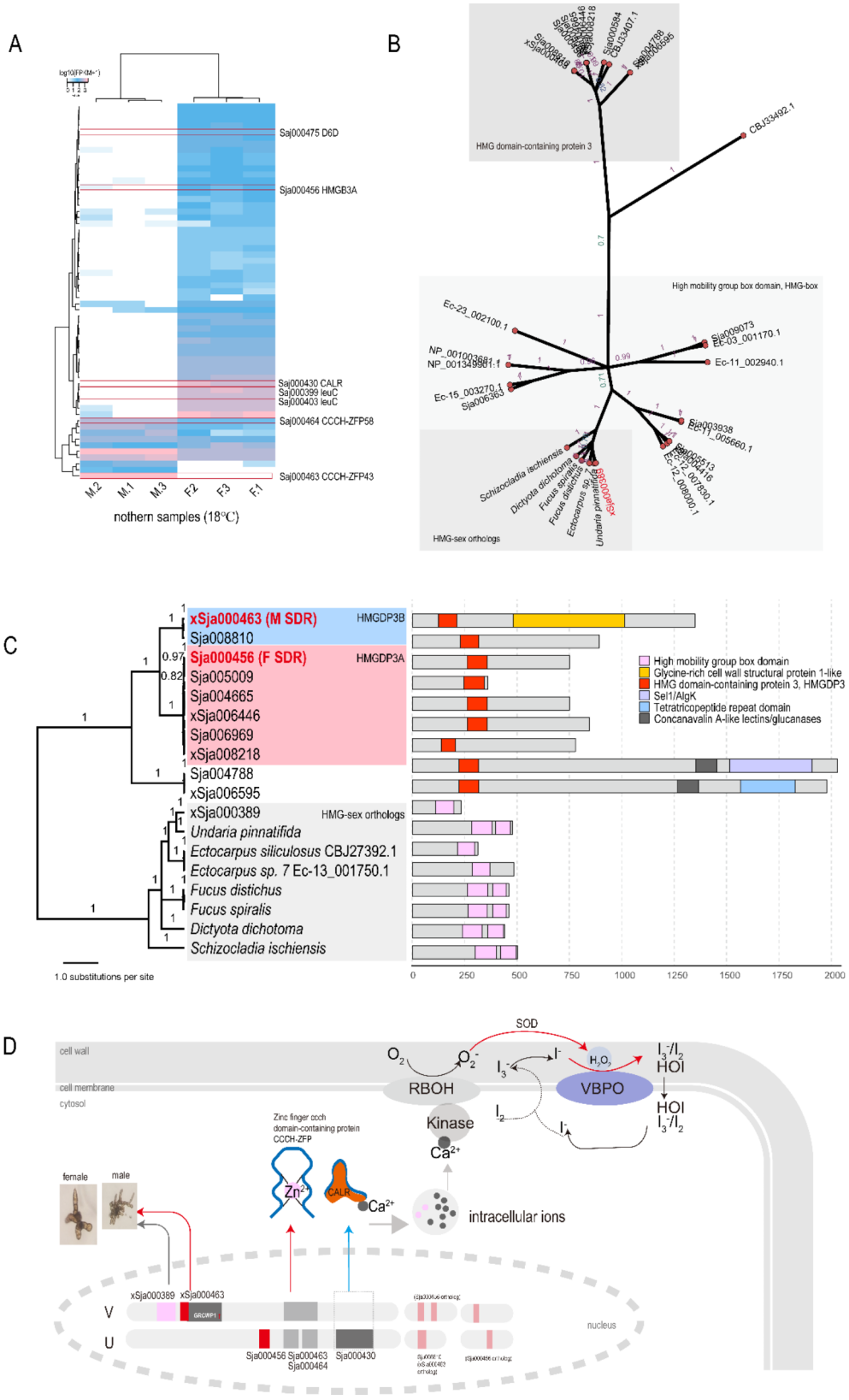

Fig. 4 (See legend on next page.)

(See figure on previous page.)

Fig. 4 Retrospective and Prospective function analysis of *S. japonica* SDR. **A**, the heatmap of the differentially expressed genes located in the SDR. The genes with identifier and demarcated with red lines both sides are those expressed at all tested conditions. **B**, Bayesian phylogenetic trees of HMG-containing protein homologs among brown algae. **C**, the structure of *HMG-sex* encoded proteins and HMG domain-containing proteins identified in *Ectocarpus* and the female and male gametophytes of *S. japonica*. **D**, the deduced sex determination mechanism of *S. japonica* gametophytes, which may need the cooperation of *HMG-sex* homolog and an extra protein, the HMG domain-containing protein 3, and the higher innate immune activity of the male gametophyte than the female one due to the existence of a zinc figure domain containing protein and calcium ion buffer, calreticulin

by hydrolyzing SUMO and deconjugating SUMO in response to nutrient starvation, hypoxia, osmotic stress, DNA damage, heat shock among others [56]. The genes associated with sex in autosomes included also the ascorbate peroxidase gene (*APX*) (chromosome 10) which encoded a key antioxidant H_2O_2 scavenging enzyme [57, 58]. The gene *Sja012038* in chromosome 25 was found to have intrinsically disordered regions (IDRs). IDR mediates the interaction among proteins in cellular processes from signal transduction to liquid-liquid phase transition, modulates protein function in response to cellular stimuli, and function as entropic springs, flexible linkers or spacers among others [59]. IDR-driven functions included also moonlighting and facilitating the formation of protein–protein interaction hubs, acting as scaffolds proteinaceous machinery, facilitating alternative splicing and posttranslational modifications among others [60]. From autosomes to sex chromosomes and finally within SDR, it seemed that many genes involved in diverse traits, especially stress response, are merging.

About 186 genes were found in the SDR of V chromosome and 134 gene in the SDR of U chromosome (Fig. 3F), which are more than those in the SDR of *Ectocarpus*. Except for gene content, the gene type between *S. japonica* and *Ectocarpus* is also extremely different. Only a few homologous gene were found between them, which included *HMG-sex*, the master determiner of male gametophytes [15].

In total, 62 genes expressed differentially between northern male and northern female gametophytes, 10 up-regulated and 52 down-regulated (Fig. 4A). When northern and southern gametophytes were shifted from18℃ to 23℃, 107 differentially expressed genes (DEGs) showed a similar trend of either upregulation or downregulation, of them, 36 locate in the SDR. Such proportion (36/107, 33.6%) implied that the high temperature associating genes have become the sex-linked. Among 36 sex-linked genes, 8 were functionally annotated. In the SDR of *S. japonica*, only 7 genes were found to have homologs in the SDR of *Ectocarpus* [14]. The traits (phenotypes) controlled by the genes in SDR are different substantially between *S. japonica* and *Ectocarpus* except for the sex (gender) itself. A set of proteins containing either HMG-box domain or HMG domain were annotated, of them, only HMG-sex homolog was found to have HMG-box domain, which locates in the V chromosome of *S. japonica* male gametophyte while others have HMG domain (Fig. 4B). In addition, two HMG domain-containing protein 3 (MGDP3) genes, *HMGDP3A* and *HMGDP3B*, were found to be specific for *S. japonica*. Two *HGMB3A* located in an autosome and the SDR of U chromosome, respectively, while one *HMGDP3B* located in the SDR of V chromosome. The former expressed highly in female gametophyte while the latter expressed highly in male gametophyte. The function of HMGDP3A and HMGDP3B is not clear; however, the latter fused a domain, glycine-rich cell wall structural protein 1-like (aa483-aa1014), which highly expressed in female gametophyte, and may associate with the morphological difference between male and female gametophytes [61]. Glycine-rich proteins have been found to work in the stress tolerance of plant [62, 63] and pollen development and fertility performance [64] (Fig. 4C).

The genes in the SDR *S. japonica* V chromosome included also the zinc finger ccch domain-containing protein and calreticulin (CALR) genes, which was up- and down-regulated in male gametophyte, respectively. Diverse zinc-finger domains make zinc finger proteins able to interact with DNA, RNA and other proteins, regulate many cellular processes including plant abiotic stress tolerance [65, 66]. Cellular repertoire of zinc homeostatic molecules includes zinc-binding proteins, transporters and zinc ion sensors [67]. Except for as the regulators, zinc figure proteins may play a role in stabilizing cellular zinc ion content. Up-regulating the expression of zinc finger ccch domain-containing protein gene should reduce the cellular zinc ion concentration, causing the relative increase of cellular calcium ion content [68]. Simultaneously down-regulating the expression of *CALR* should further increase the calcium ion content [69]. The increase of calcium ion will initiate the kinases to activate RBOH, yielding more H_2O_2 with the help of SOD. Up-regulated SOD and VBPO genes intensify the innate immune system and iodine cycle, an energy exhausting process we have proposed (Fig. 4D).

Discussion

Sex determination mechanism of *S. japonica*

S. japonica and *Ectocarpus* determine the gender of their gametophytes in a similar way. They share the master sex determiner, *HMG-sex* and a few homologous genes. The sex determination is evolving, and not yet evolved. A few genes located on the autosomes rather than U and

V chromosomes, and a few genes located outside SDR. Except for sex trait itself, these two brown algae are obviously different in morphology and inhabitation environment, which may have caused the differences in the number and type of the sex associated, sex-linked and SDR-hold genes. In fact, the number of genes in *S. japonica* SDR was more than that in *Ectocarpus* SDR (>100 vs. ~20). The physiological processes these genes involved are not completely clear. In this study, we found that the sex associated genes of *S. japonica* on autosomes are stress tolerance related, and at least two genes in its SDR function in the innate immune system, thus heat stress tolerance related. Except for sex trait itself, the genes merged into SDR and becoming sex associated are stress tolerance related. The sex determination may evolve in three phases, initiation with the master determination gene, gradual integration of stress tolerance genes into SDR, and establishment of complete sex chromosome.

Even the sex trait itself can be reversed by both extrinsic and intrinsic fuses, for example, temperature and hormones. It has been documented that the brown algal sex is reversable [70]. For brown algae, the sex determination may be simply described as that a gene or a few genes determine the differential development of gametes and gametophytes, which then become linked or associated with the stress responding/ tolerating genes, favoriting the survival and evolution of an algal species.

Genetically, the SDR in V chromosome of *S. japonica* male gametophyte has integrated the stress tolerance genes. Physiologically, the male gametophytes showed stronger PEP-pyruvate-oxaloacetate node exhausting power than the female gametophytes. The brown algal gametophytes share their gender determination mechanisms with animals [15]. Temperature influences the male fertility of animals [48, 71] and plants [72], and organism's survival [73]. The male gametophytes are stronger than female in heat tolerance; however, the female gametophytes should determine the ecological niche and distribution of *S. japonica*.

The sex-linked or sex-associated genes primarily involve in sex determination and sex cell production; however, accumulating evidences suggest that these genes also control the traits not sex-limited or directly involved in reproduction. Diverse non-reproductive traits are affected by sex-limited chromosomes in humans, which include, for example, a group of non-sexual traits affected by Y chromosome [74], differential stress responses related to genes on the sex chromosomes [75], different diseases involving oxidative and proteolytic stresses [76], and biased immunities [77]. The copepod *Tigriopus californicus* does not have sex chromosomes. However, its tolerances to a range of acute stressors like high temperature, high salinity, low salinity, copper and bisphenol A are sexually dimorphic [78, 79]. The old fruit fly females are more stress tolerant than the males [80]. In tropical liverwort, *Marchantia infexa*, two dehydration associating genes are found to be sex-linked [81]. Among dioecious plants, various vegetative growth and stress tolerance differentials have been identified to be sex-related, which include water stress, elevated temperatures, cold temperatures, chilling, altitudinal gradient among others [82]. Among the genes located in the sex determination regions (SDRs) of the U and V chromosomes and those associated with sex but not located in the SDRs of kelp gametophytes, we identified the genes which may function in responding the relative high seawater temperature, extended their function to iodine cycling and thermo-exhaustion, and finally ligated them with high temperature resistance. We proposed also that the tolerance relating genes become either linked to or associated with the master sex controlling gene(s) as the mechanism underlining the sex evolution among kelp gametophytes. To some extent, this is a new understanding about the sex determination and sex evolution among brown algae including *S. japonica*.

The conflict between growth performance and stress defense

Resistance/ tolerance to diverse stresses accompany always the attenuation of vegetative growth of plants [83]. Such spell can be broken by hormones (plant peptides) [84]. The sporophyte of kelp is harvested for different purposes ahead of sporangium development. The cultivated kelp may not have to solve the conflict between defense and growth. However, for the cultivated kelp to revert to wild, a balance between the growth of both sporophyte and gametophyte and survival (defense) must be realized. This restriction may have determined the small sporophyte size of the wild kelp sporophytes frequently documented in northern cultivation area.

We have found that southern kelp cultivars have adapted northern sea areas (18℃), and acclimated southern sea area (23℃) by adjusting many metabolism routes. However, no adaptation and acclimation routes were found to associate with the growth performance of gametophytes, implying that the kelp has not yet overcome the conflict between growth and defense. The global change has caused diversity loss of macroalgae [11]. Thermal refuges may reinforce macroalgae survive the climate change [85]. Artificial domestication/ cultivation of kelp may serve as an additional method of conserving the macroalgal diversity and saving some macroalgal species even.

Comparative studies may aid to understanding the mechanism of heat tolerance

As the brown algae, some species survive and grow well in the seawater with high temperature. For example,

pelagic *Sargassum* is abundant in the Sargasso Sea, and has been found to form a great Atlantic *Sargassum* belt (GASB) often [86]. The climate change may have aided to the extraordinary proliferation of *Sargassum* in the tropical Atlantic [87]. The draft genome assemblies of two *Sargassum* species have become available currently [88, 89]. The comparison between *Sargassum* and *Saccharina* genomes may provide clues deepening our understanding the high seawater temperature tolerance mechanism of kelp.

The genome of *Saccharina japonica* was sequenced once with sporophytic DNA and assembled from both short and long reads generated on the second and third generation sequencing platforms [90]. The sporophyte of *S. japonica* is diploidy; thus, the genome assembly is chimeric, not haplotypic, making the comparison between the sex determination region in structure and gene component impossible. Our assemblies are sex-separated, which should aid to detailing the comparison between male and female genders. In fact, we have compared the structure and gene component of the SDRs located on U and V chromosomes. We believe that our assemblies should be more referable in diverse genetic studies. In combination with the GWAS location of SDRs, we have also bounded the SRDs of kelp gametophytes genetically for the first time.

Comparison among brown algal genomes has revealed that emergence of the brown algae is associated with the gain of new genes, protein domain rearrangement, horizontal gene transfer and the novel signaling molecules and key metabolic pathways, and the brown algal genome diversification parallels the phenotypic divergence [91]. Besides these common characteristics, jointly comparing the genomic and transcriptomic differences may aid to unveiling those subtle but critical differentials implying the genetic mechanisms of important traits.

The complex heat defense trait may determine the ecological distribution of a species

The response of organisms to climate change may bias the sex ratio at the individual, population and community levels [17, 92]. The mechanisms to thermal, saline-alkali, cold, low nutritional tolerances of organisms are diverse, and the tolerance traits are complex. Among diverse stresses, the thermal one may have played a crucial role in determining the global distribution of species. Even the rice with equatorial habitats may shift its habitats to higher latitudes and mountains with higher altitudes [93]. As the leading abiotic factor, temperature may have influenced the genome diversity of rice and its dispersal trajectory in Asia [94]. For *S. japonica*, seawater temperature should modulate its distribution.

The putative controlling genes of the tolerance to relative high seawater temperature

We have carried out both GWAS and transcriptomic analysis to identify the genes which may control the high-water temperature adaptation trait. We have found a set of intersecting genes with GWAS and transcriptomic analysis, respectively, which include but not limit to vanadium-dependent bromoperoxidase, superoxide dismutase among others.

The SNPs located at either the downstream or upstream of the genes identified with GWAS, and sometime the SNPs located in the genes (genic). The genes differentially expressed were identified according to the abundance of their transcripts. It is hard to explain the differential expression of genes with the SNPs around them. We are waiting for the methodological advancements like the genome edition, antisense RNA, genetic transformation among others which are applicable for *S. japonica*, and convenient and highly efficient in operation. Once the methods like CRISPR and genetic transformation (critical to gene knock out, over expression, gene knock down among others) are implemented for kelp, and become maneuverable, we will verify the function of the genes responding to relative high temperature stress. In fact, these novel methods are brewing [95–101], and we believe that they will come into being soon. Simultaneously, we appreciate also our fellows and colleagues to verify the functions of these genes.

Conclusions

We have isolated sets of kelp gametophytes representing the kelp varieties cultivated in northern cultivation region (Liaoning and Shandong) and southern cultivation region (Fujian). To provide a high-quality reference genome, we sequenced the gametophyte genome, both female and male, and obtained the chromosomal level assemblies. From Dalian to Fujian, the lowest and the highest seawater temperatures in the period of cultivation increased by 14℃and 8℃, respectively, which provided the appropriate materials for deciphering the adaptation and acclimation of kelp to relative high seawater temperature. We found that the gametophytes of southern China kelp cultivation area adapted the high seawater temperature by transforming amino acids, changing sugar metabolism and intensifying innate immune system, and acclimating to the southern China seawater temperature by transforming amino acids, changing sugar metabolism to the glycosylation of protein and osmotic pressure maintenance, intensifying the innate immune system, and exhausting energy and reduction power through the PEP-pyruvate-oxaloacetate node and the iodine cycle. The sex of *S. japonica* was determined by *HMG-sex* as *Ectocarpus* does. During the evolution of sex trait, the stress responding/ tolerating genes become

linked to or associated with this master sex determiner. The male gametophyte was stronger than the female in responding the relative high seawater temperature. We should recognize that elevated high temperature tolerance of kelp may enable it to grow cross summer season and make it become an invading species finally. This is an issue we should focus on and carefully examine in future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11382-7.

Supplementary Material 1

Supplementary Material 2

Author contributions

L.G. processed data; X. L. prepared and maintained materials; S.C., Y.L., W.W., S.L., L.J. participated in preparing and maintaining materials; F.L., L.Z., G.B., discussed the experimental design and hypothesized ideas; H.L., X.P., Y.Z. L.F., participated in processing data; G.Y., conceived and organized project, interpreted results and first drafted manuscript.

Funding

This work was financially supported by the Major Scientific and Technological Innovation Project of Shandong Provincial Key Research and Development Program (2022LZGC004); the Science & Technology Innovation Project of Laoshan Laboratory (LSKJ202203205); the Earmarked Fund for CARS (CARS-50); the Fundamental Research Funds for the Central Universities (202262001); National Key R & D Program of China (2022YFF1102300, 2018YFD0901500); and Modern Agricultural Technology Industry System of Shandong Province (SDAIT-26-02); and National Natural Science Foundation of China (32102757; 32072962).

Data availability

The whole genome sequence data reported in this paper have been deposited in the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics, Chinese Academy of Sciences, China National Center for Bioinformation, under accession numbers GWHBJIV00000000 (the female gametophyte of kelp, Saccharina japonica) and GWHFLAL00000000.1 (the male gametophyte of kelp) that are publicly accessible at https://ngdc.cncb.ac.cn/gwh. The raw sequences data associating with these two kelp gametophyte genome assemblies have been deposited in National Center for Biotechnology Information (NCBI), and are accessible at https://www.ncbi.nlm.nih.gov/sra under the accession numbers SRX9668532 (PACBIO reads, male), SRX9645476 (Illumina reads, male), SRX9645565 (Hi-C reads, male), SRX9038829 (PACBIO reads, female), SRX9038689 (Illumina reads, female), SRX9048964 (Hi-C reads, female), and SRX9038827 (RNA reads). These raw sequences are also seeable in bulk at https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all%26;from_uid=660288.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 11 December 2024 / Accepted: 19 February 2025
Published online: 24 February 2025

References

1. Bartsch I, Wiencke C, Bischof K, Buchholz CM, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, et al. The genus *Laminaria sensu lato*: recent insights and developments. Eur J Phycol. 2008;43(1):1–86.
2. Lane CE, Mayes C, Druehl LD, Saunders GW. A multi-gene molecular investigation of the Kelp (Laminariales, Phaeophyceae) supports substantial taxonomic re-organization. J Phycol. 2006;42(4):962–962.
3. Zhang J, Wang X, Yao J, Li Q, Liu F, Yotsukura N, Krupnova TN, Duan D. Effect of domestication on the genetic diversity and structure of *Saccharina Japonica* populations in China. Sci Rep. 2017;7:42158.
4. Hu ZM, Shan TF, Zhang QS, Liu FL, Jueterbock A, Wang GG, Sun ZM, Wang XY, Chen WZ, Critchley AT, Ye NH. Kelp breeding in China: challenges and opportunities for solutions. Reviews Aquaculture. 2024;16:855–71.
5. Hu ZM, Shan TF, Zhang J, Zhang QS, Critchley AT, Choi HG, Yotsukura N, Liu FL, Duan DL. Kelp aquaculture in China: A retrospective and future prospects. Reviews Aquaculture. 2021;13:1324–51.
6. Li X, Cong Y, Yang G, Shi Y, Qu S, Li Z, Wang G, Zhang Z, Luo S, Dai H, et al. Trait evaluation and trial cultivation of Dongfang 2, the hybrid of a male gametophyte clone of *Laminaria longissima* (Laminariales, Phaeophyta) and a female one of *L. japonica*. J Appl Phycol. 2007;19(2):139–51.
7. Li X, Wang Y, Xiao L, Huang E, Liu X, Shan T. Pang. *Saccharina* sporophytes settling on Kelp cultivation rafts come from wild intertidal populations and not from the farmed cultivars. Bot Mar. 2024;67:231–8.
8. Feng X, Li H, Zhang Z, Xiong T, Shi X, He C, Shi Q, Jiao N, Zhang Y. Microbial-mediated contribution of Kelp detritus to different forms of oceanic carbon sequestration. Ecol Indic. 2022;142:109186.
9. Li H, Zhang Z, Xiong T, Tang K, He C, Shi Q, Jiao N, Zhang Y. Carbon sequestration in the form of recalcitrant dissolved organic carbon in a seaweed (kelp) farming environment. Environ Sci Technol. 2022;56(12):9112–22.
10. Li X, Zhang Z, Qu S, Liang G, Zhao N, Sun J, Song S, Cao Z, Li X, Pan J, et al. Breeding of an intraspecific Kelp hybrid Dongfang 6 (*Saccharina japonica*, phaeophyceae, Laminariales) for suitable processing products and evaluation of its culture performance. J Appl Phycol. 2016;28(1):439–47.
11. Manca F, Benedetti-Cecchi L, Bradshaw CJA, Cabeza M, Gustafsson C, Norkko AM, Roslin TV, Thomas DN, White L, Strona G. Projected loss of brown macroalgae and seagrasses with global environmental change. Nat Commun. 2024;15(1):5344.
12. Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman TL, Hahn MW, Kitano J, Mayrose I, Ming R, et al. Sex determination: why so many ways of doing it?? Plos Biol. 2014;12(7):e1001899.
13. Coelho SM. The brown seaweed *Ectocarpus*. Nat Methods. 2024;21(3):363–4.
14. Ahmed S, Cock JM, Pessia E, Luthringer R, Cormier A, Robuchon M, Sterck L, Peters AF, Dittami SM, Corre E, et al. A haploid system of sex determination in the brown Alga *Ectocarpus* Sp. Curr Biol. 2014;24(17):1945–57.
15. Luthringer R, Raphalen M, Guerra C, Colin S, Martinho C, Zheng M, Hoshino M, Badis Y, Lipinska AP, Haas FB, et al. Repeated co-option of HMG-box genes for sex determination in brown algae and animals. Science. 2024;383(6689):eadk5466.
16. Vigneau J, Martinho C, Godfroy O, Zheng M, Haas FB, Borg M, Coelho SM. Interactions between U and V sex chromosomes during the life cycle of *Ectocarpus*. Development. 2024;151(20):dev202677.
17. Gissi E, Schiebinger L, Hadly EA, Crowder LB, Santoleri R, Micheli F. Exploring climate-induced sex-based differences in aquatic and terrestrial ecosystems to mitigate biodiversity loss. Nat Commun. 2023;14(1):4787.
18. Valenzuela N, Literman R, Neuwald JL, Mizoguchi B, Iverson JB, Riley JL, Litzgus JD. Extreme thermal fluctuations from climate change unexpectedly accelerate demographic collapse of vertebrates with temperature-dependent sex determination. Sci Rep. 2019;9:4254.
19. Lockley EC, Eizaguirre C. Effects of global warming on species with temperature-dependent sex determination: bridging the gap between empirical research and management. Evol Appl. 2021;14(10):2361–77.
20. Zhang L, Cui C, Li X, Zhang Z, Luo S, Liang G, Liu Y, Yang G. Effect of temperature on the development of *Saccharina Japonica* gametophytes. J Appl Phycol. 2013;25(1):261–7.
21. Zhang L, Cui C, Li Y, Wu H, Li X. A genome screen for the development of sex-specific DNA markers in *Saccharina japoni*ca. J Appl Phycol. 2018;30(2):1239–46.
22. Thomas F, Cosse A, Le Panse S, Kloareg B, Potin P, Leblanc C. Kelps feature systemic defense responses: insights into the evolution of innate immunity in multicellular eukaryotes. New Phytol. 2014;204(3):567–76.

23. Rivas FJM, Fernie AR, Aarabi F. Roles and regulation of the RBOHD enzyme in initiating ROS-mediated systemic signaling during biotic and abiotic stress. Plant Stress. 2024;11:100327.
24. Küpper FC, Carrano CJ. Key aspects of the iodine metabolism in brown algae: a brief critical review. Metallomics. 2019;11(4):756–64.
25. Mazéas L, Yonamine R, Barbeyron T, Henrissat B, Drula E, Terrapon N, Nagasato C, Hervé C. Assembly and synthesis of the extracellular matrix in brown algae. Semin Cell Dev Biol. 2023;134:112–24.
26. Mickelbart MV, Hasegawa PM, Bailey-Serres J. Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. Nat Rev Genet. 2015;16(4):237–51.
27. Song X, Wang J, Sun P, Ma X, Yang Q, Hu J, Sun S, Li Y, Yu J, Feng S, et al. Preferential gene retention increases the robustness of cold regulation in Brassicaceae and other plants after polyploidization. Hortic Res. 2020;7(1):20.
28. Zhou X, Joshi S, Patil S, Kharem T, Kumar V. Reactive oxygen, nitrogen, carbonyl and sulfur species and their roles in plant abiotic Atress responses and tolerance. J Plant Growth Regul. 2022;41:119–42.
29. Hasanuzzaman M, Bhuyan MHMB, Anee TI, Parvin K, Nahar K, Al Mahmud J, Fujita M. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants-Basel. 2019;8(9):384.
30. Cheng M, Ko K, Chang W, Kuo W, Chen G, Lin T. Increased glutathione contributes to stress tolerance and global translational changes in Arabidopsis. Plant J. 2015;83(5):926–39.
31. Raza A, Bhardwaj S, Rahman MA, García-Caparrós P, Habib M, Saeed F, Charagh S, Foyer CH, Siddique KHM, Varshney RK. Trehalose: A sugar molecule involved in temperature stress management in plants. Crop J. 2024;12(1):1–16.
32. Takahashi H, Marsolais F, Cuypers A, Kopriva S. Sulfur metabolism: actions for plant resilience and environmental adaptation. J Exp Bot. 2023;74(11):3271–5.
33. Bröer S, Bröer A. Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem J. 2017;474:1935–63.
34. Koendjbiharie JG, van Kranenburg R, Kengen SWM. The PEP-pyruvate-oxaloacetate node: variation at the heart of metabolism. FEMS Microbiol Rev. 2021;4:fuaa061.
35. Sauer U, Eikmanns BJ. The PEP-pyruvate-oxaloacetate node as the switch point for carbon flux distribution in bacteria: we dedicate this paper to Rudolf K. Thauer, director of the Max-Planck-Institute for terrestrial microbiology in Marburg, Germany, on the occasion of his 65th birthday. FEMS Microbiol Rev. 2005;29:765–94.
36. Zhang H, Zhao Y, Zhu JK. Thriving under stress: how plants balance growth and the stress response. Dev Cell. 2020;55(5):529–43.
37. Edwards SV. Genomics of adaptation and acclimation: from field to lab and back. Natl Sci Rev. 2020;7(1):128.
38. Armarego-Marriott T. Climatic selection and gene expression plasticity. Nat Clim Chang. 2021;11:4.
39. Bolik S, Albrieux C, Schneck E, Demé B, Jouhet J. Sulfoquinovosyldiacylglycerol and phosphatidylglycerol bilayers share biophysical properties and are good mutual substitutes in photosynthetic membranes. Bba-Biomembranes. 2022;1864(12):184037.
40. Harwood JL, Okanenko AA. Sulphoquinovosyl Diacylglycerol (SQDG) - the sulpholipid of higher plants. In: Abrol YP, Ahmad A, editors. Sulphur in plants. Dordrecht: Springer; 2003. pp. 189–219.
41. Liu C, Niu G, Li X, Zhang H, Chen H, Hou D, Lan P, Hong Z. Comparative label-free quantitative proteomics analysis reveals the essential roles of N-Glycans in salt tolerance by modulating protein abundance in *Arabido*psis. Front Plant Sci. 2021;12:646425.
42. Cao L, Diedrich JK, Ma Y, Wang N, Pauthner M, Park SKR, Delahunty CM, McLellan JS, Burton DR, Yates JR, et al. Global site-specific analysis of glycoprotein N-glycan processing. Nat Protoc. 2018;13(6):1196–212.
43. Zhang H, Yu F, Xie P, Sun S, Qiao X, Tang S, Chen C, Yang S, Mei C, Yang D, et al. A Gγ protein regulates alkaline sensitivity in crops. Science. 2023;379(6638):eade8416.
44. Ganapati RK, Naveed SA, Zafar S, Wensheng W, Jianlong XU. Saline-alkali tolerance in rice: physiological response, molecular mechanism, and QTL identification and application to breeding. Rice Sci. 2022;29(5):412–34.
45. Gharib G, Saeidiharzand S, Sadaghiani AK, Kosar A. Antifreeze proteins: A Tale of evolution from origin to energy applications. Front Bioeng Biotech. 2022;9:770588.
46. Bennett JM, Sunday J, Calosi P, Villalobos F, Martínez B, Molina-Venegas R, Araújo MB, Algar AC, Clusella-Trullas S, Hawkins BA, et al. The evolution of critical thermal limits of life on Earth. Nat Commun. 2021;12(1):1198.
47. Lancaster LT, Humphreys AM. Global variation in the thermal tolerances of plants. P Natl Acad Sci USA. 2020;117(24):13580–7.
48. Kurhanewicz NA, Dinwiddie D, Bush ZD, Libuda DE. Elevated temperatures cause transposon-associated DNA damage in *C. elegans* spermatocytes. Curr Biol. 2020;30(24):5007–17.
49. Hirano K, Nonami Y, Nakamura Y, Sato T, Sato T, Ishiguro K, Ogawa T, Yoshida S. Temperature sensitivity of DNA double-strand break repair underpins heat-induced meiotic failure in mouse spermatogenesis. Commun Biol. 2022;5(1):504.
50. Uffelmann E, Huang Q, Munung NS, de Vries J, Okada Y, Martin AR, Martin HC, Lappalainen T, Posthuma D. Genome-wide association studies. Nat Rev Method Prime. 2021;1(1):59.
51. Zhu Z, Younas L, Zhou Q. Evolution and regulation of animal sex chromosomes. Nat Rev Genet. 2024. https://doi.org/10.1038/s41576-024-00757-3.
52. Abbott JK, Nordén AK, Hansson B. Sex chromosome evolution: historical insights and future perspectives. P Roy Soc B-Biol Sci. 2017;284(1854):20162806.
53. Coelho SM, Gueno J, Lipinska AP, Cock JM, Umen JG. UV chromosomes and haploid sexual systems. Trends Plant Sci. 2018;23(9):794–807.
54. Vigneau J, Martinho C, Godfroy O, Zheng M, Haas FB, Borg M, Coelho SM. Interactions between U and V sex chromosomes during the life cycle of *Ectocarpus*. Development. 2024;151:dev202677.
55. Coelho SM, Mignerot L, Cock JM. Origin and evolution of sex-determination systems in the brown algae. New Phytol. 2019;222(4):1751–6.
56. Peek J, Harvey C, Gray D, Rosenberg D, Kolla L, Levy-Myers R, Yin R, McMurry JL, Kerscher O. SUMO targeting of a stress-tolerant Ulp1 SUMO protease. PLoS ONE. 2018;13(1):e0191391.
57. Pandey S, Fartyal D, Agarwal A, Shukla T, James D, Kaul T, Negi YK, Arora S, Reddy MK. Abiotic stress tolerance in plants: myriad roles of ascorbate peroxidase. Front Plant Sci. 2017;8:581.
58. Li S. Novel insight into functions of ascorbate peroxidase in higher plants: more than a simple antioxidant enzyme. Redox Biol. 2023;64:102789.
59. Piovesan D, Necci M, Escobedo N, Monzon AM, Hatos A, Micetic I, Quaglia F, Paladin L, Ramasamy P, Dosztányi Z, et al. MobiDB: intrinsically disordered proteins in 2021. Nucleic Acids Res. 2021;49(D1):D361–7.
60. Kurgan L, Hu G, Wang K, Ghadermarzi S, Zhao B, Malhis N, Erdos G, Gsponer J, Uversky VN, Dosztányi Z. Tutorial: a guide for the selection of fast and accurate computational tools for the prediction of intrinsic disorder in proteins. Nat Protoc. 2023;18(11):3157–72.
61. Ringli C, Keller B, Ryser U. Glycine-rich proteins as structural components of plant cell walls. Cell Mol Life Sci. 2001;58(10):1430–41.
62. Yu W, Dai Y, Chen J, Liang A, Wu Y, Suo Q, Chen Z, Yan X, Wang C, Lai H, et al. Upregulation of the glycine-rich protein-encoding gene GhGRPL enhances plant tolerance to abiotic and biotic stressors by promoting secondary cell wall development. J Integr Agr. 2024;23(10):3311–27.
63. Czolpinska M, Rurek M. Plant glycine-rich proteins in stress response: an emerging, still prospective story. Front Plant Sci. 2018;9:302.
64. Takebe N, Nakamura A, Watanabe T, Miyashita A, Satoh S, Iwai H. Cell wall glycine-rich protein 2 is involved in tapetal differentiation and pollen maturation. J Plant Res. 2020;133(6):883–95.
65. Cassandri M, Smirnov A, Novelli F, Pitolli C, Agostini M, Malewicz M, Melino G, Raschellà G. Zinc-finger proteins in health and disease. Cell Death Discov. 2017;3:17071.
66. Han G, Qiao Z, Li Y, Wang C, Wang B. The roles of CCCH zinc-finger proteins in plant abiotic stress tolerance. Int J Mol Sci. 2021;22(15):8327.
67. Colvin RA, Holmes WR, Fontaine CP, Maret W. Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. Metallomics. 2010;2(5):306–17.
68. Hershfinkel M. Cross-talk between zinc and calcium regulates ion transport: A role for the zinc receptor, ZnR/GPR39. J Physiol. 2024;602(8):1579–94.
69. Fucikova J, Spisek R, Kroemer G, Galluzzi L. Calreticulin and cancer. Cell Res. 2021;31(1):5–16.
70. Müller DG, Gaschet E, Godfroy O, Gueno J, Cossard G, Kunert M, Peters AF, Westermeier R, Boland W, Cock JM, et al. A partially sex-reversed giant Kelp sheds light into the mechanisms of sexual differentiation in a UV sexual system. New Phytol. 2021;232(1):252–63.
71. Gandara ACP, Drummond-Barbosa D. Chronic exposure to warm temperature causes low sperm abundance and quality in. Sci Rep. 2023;13(1):12331.
72. Li XL, Bruckmann A, Dresselhaus T, Begcy K. Heat stress at the bicellular stage inhibits sperm cell development and transport into pollen tubes. Plant Physiol. 2024;195(3):2111–28.

73. Wang W, Gunderson AR. The physiological and evolutionary ecology of sperm thermal performance. Front Physiol. 2022;13:754830.
74. Cīrulis A, Hansson B, Abbott JK. Sex-limited chromosomes and non-reproductive traits. BMC Biol. 2022;20(1):156.
75. Bale TL, Epperson CN. Sex differences and stress across the lifespan. Nat Neurosci. 2015;18(10):1413–20.
76. Tower J, Pomatto LCD, Davies KJA. Sex differences in the response to oxidative and proteolytic stress. Redox Biol. 2020;31:101488.
77. Feng Z, Liao M, Zhang L. Sex differences in disease: sex chromosome and immunity. J Translational Med. 2024;22(1):1150.
78. Foley HB, Sun PY, Ramirez R, So BK, Venkataraman YR, Nixon EN, Davies KJA, Edmands S. Sex-specific stress tolerance, proteolysis, and lifespan in the invertebrate *Tigriopus californicus*. Exp Gerontol. 2019;119:146–56.
79. Li N, Flanagan BA, Edmands S. Food deprivation exposes sex- specific trade- offs between stress tolerance and life span in the copepod *Tigriopus californicus*. Ecol Evol. 2022;12:e8822.
80. Kristensen TN, Loeschcke V, Tan Q, Pertoldi C, Mengel-From J. Sex and age specific reduction in stress resistance and mitochondrial DNA copy number in *Drosophila melanogaster*. Sci Rep. 2019;9(1):12305.
81. Marks RA, Smith JJ, Cronk Q, Grassa CJ, McLetchie DN. Genome of the tropical plant *Marchantia inflexa*: implications for sex chromosome evolution and dehydration tolerance. Sci Rep. 2019;9(1):8722.
82. Juvany M, Munné-Bosch S. Sex-related differences in stress tolerance in dioecious plants: A critical appraisal in a physiological context. J Exp Bot. 2015;66(20):6083–92.
83. Zhang H, Zhao Y, Zhu JK. Thriving under stress: how plants balance growth and the stress response. Dev Cell. 2020;55:529–43.
84. Lin ZB, Zhao YX, Yang WC. A balanced mechanism between plant growth and stress response. Adv Agrochem. 2023;2:105–6.
85. Arriaga O, Wawrzynkowski P, Muguerza N, Díez I, González J, Gorostiaga JM, Quintano E, Becerro MA. Thermal refugia reinforce macroalgal resilience against climate change in the southeastern Bay of Biscay. Global Change Biol. 2024;30(8):e17481.
86. Wang M, Hu C, Barnes BB, Mitchum G, Lapointe B, Montoya JP. The great Atlantic Sargassum belt. Science. 2019;365(6448):83–7.
87. Marsh R, Skliris N, Tompkins EL, Dash J, Almela VD, Tonon T, Oxenford HA, Webber M. Climate-*sargassum* interactions across scales in the tropical Atlantic. PloS Clim. 2023;2:e0000253.
88. Wang S, Wu M. The draft genome of the golden tide seaweed, Sargassum Horneri: characterization and comparative analysis. Genes (Basel). 2023;14(10):1969.
89. Wang S, Lin L, Shi Y, Qian W, Li N, Yan X, Zou H, Wu M. First draft genome assembly of the seaweed Sargassum fusiforme. Front Genet. 2020;11:590065.
90. Ye N, Zhang X, Miao M, Fan X, Zheng Y, Xu D, Wang J, Zhou L, Wang D, Gao Y, et al. *Saccharina* genomes provide novel insight into Kelp biology. Nat Commun. 2015;6:6986.
91. Denoeud F, Godfroy O, Cruaud C, Heesch S, Nehr Z, Tadrent N, Couloux A, Brillet-Guéguen L, Delage L, Mckeown D, et al. Evolutionary genomics of the emergence of brown algae as key components of coastal ecosystems. Cell. 2024;187(24):6943–e696539.
92. Valenzuela N, Literman R, Neuwald JL, Mizoguchi B, Iverson JB, Riley JL, Litzgus JD. Extreme thermal fluctuations from climate change unexpectedly accelerate demographic collapse of vertebrates with temperature-dependent sex determination. Sci Rep. 2019;9(1):4254.
93. Lin Y, Chen Y, Wang H, Hong J, Yang R, Cao Y, Yan S, Fang W. Global potential distributions and conservation status of rice wild relatives. Plants People Planet. 2024;6(4):979–91.
94. Gutaker RM, Groen SC, Bellis ES, Choi JY, Pires IS, Bocinsky RK, Slayton ER, Wilkins O, Castillo CC, Negrão S, Oliveira MM, Fuller DQ, Guedes JAD, Lasky JR, Purugganan MD. Genomic history and ecology of the geographic spread of rice. Nat Plants. 2020;6(5):492–502.
95. de Saeger J, Coulembier-Vandelannoote E, Lee H, Park J, Blomme J. Genome editing in macroalgae: advances and challenges. Front Genome Ed. 2024;6:1380682.
96. Shen Y, Motomura T, Ichihara K, Matsuda Y, Yoshimura K, Kosugi C, Nagasato C. Application of CRISPR-Cas9 genome editing by microinjection of gametophytes of *Saccharina Japonica* (Laminariales, Phaeophyceae). J Appl Phycol. 2023;35:1431–41.
97. Deng X, Qin S, Zhang Q, Jiang P, Cui Y, Li X. Microprojectile bombardment of *Laminaria Japonica* gametophytes and rapid propagation of Transgenic lines within a bubble-column bioreactor. Planr Cell Tissue Organ Cult. 2009;97:253–61.
98. Li F, Qin S, Jiang P, Wu Y, Zhang W. The integrative expression of GUS gene driven by FCP promoter in the seaweed *Laminaria Japonica* (Phaeophyta). J Appl Phycol. 2009;21:287–93.
99. Jiang P, Qin S, Tseng CK. Expression of the LacZ reporter gene in sporophytes of the seaweed *Laminaria Japonica* (Phaeophyceae) by gametophyte targeted transformation. Plant Cell Rep. 2003;21(12):1211–6.
100. Jiang P, Qin S, Tseng C. Expression of hepatitis B surface antigen gene (*HBsAg*) in *Laminaria Japonica* (Laminariales, Phaeophyta). Chin Sci Bull. 2002;47:1438–40.
101. Qin S, Jiang P, Li X, Wang X, Zeng CK. A transformation model for *Laminaria Japonica* (Phaeophyta, Laminariales. Chin J Oceanol Limnol. 1998;16:50–5.

Publisher's note