Master's Thesis of Science

Effect of Temperature Stress on Recombination Frequency in Inter-varietal Hybrid Populations of Rice

February, 2020

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Effect of Temperature Stress on Recombination Frequency in Inter-varietal Hybrid Populations of Rice

UNDER THE DIRECTION OF PROFESSOR HEE-JONG KOH
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF SEOUL NATIONAL UNIVERSITY

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APPROVED AS A QUALIFIED THESIS OF YOO SEOK KANG
FOR THE DEGREE OF MASTER OF SCIENCE
BY THE COMMITTEE MEMBERS

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ABSTRACT

Inter-subspecific breeding, one of the most basic and practical methods of improvement of crop varieties, which assemble desirable alleles between them based on homologous recombination. Along with recombination, there are also existed unexpected phenotypic variation, segregation distortion. An understanding of the patterns of recombination frequency and segregation distortion is important for prediction of the phenotypic variation in the progenies. Meiotic crossover is mechanistically essential for ensuring proper chromosome segregation and offsprings gain novel combinations through recombination. Thus, recombiantion is

important factor in the aspects of life cycles and evolution. Numerous research have shown that environmental factor especially temperature, would influence on the variation of recombination. In rice, previous research already reported that temperature stress affected on recombination frequency. However, they were used only three or four samples, indicating that it needs to confirm complementary test on recombination frequency. On the other hands, relationship between temperature stress and segregation distortion are largely unknown. Here, we confirmed that recombination frequency affected along with temperature stress using up to 100 samples. Recombination frequency decreased about 4% at lower temperature and increased maximum 10% at higher temperature on chromosome level. Also, segregation distortion were alleviated at exposure of heat stress. This work would be helpful for understanding of hybrid rice breeding to predict phenotypic variation.

Key words : rice, temperature stress, recombination frequency, crossovers, segregation distortion, heat stress

Student number : 2018-23411

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LIST OF ABBREVIATIONS

NP	Nipponbare
DJ	Donjin
KS	Kasalath
INDEL	Insertion / deletion
SNP	Single nucleotide polymorphism
CO	Crossover
NCO	Non-crossover
DSB	Double strand break
ssDNA	single strand DNA
ssDNA	single strand DNA
JM	Joint molecule
SDSA	Synthesis-dependent strand annealing
MMR	Mismath repair
LND	Low nucleosome density
SD	Segregation distortion
mSD	male Segregation distortion
fSD	female Segregation distortion

INTRODUCTION

Meiotic crossover and sister chromatid cohesion mechanistically provide physical links between homologous chromosomes ensuring proper chromosome segregation during the first meiotic division(Page et al., 2003). In the evolutionary aspects, heritable variation in fitness is the fuel of adaptive evolution, and sex can generate more adaptive combinations of alleles through recombination(e.g., Barton 1995; Smith 1996; Cooper 2007). Absence of crossover leads to chromosome non-disjunction, which would produce aneuploidy resulted in gametophyte sterility (Higgins., 2004).

Metiotic recombination involves the formation and repair of programmed DSBs yielding COs or NCOs. This process is initiated by conserved Spo11 endonuclease(Bergerat., 1997). DSB end undergoes 5' to 3' resection to generate 3' single-stranded ssDNA that is catelyzed by MRX(Mre11, Rad50 and Xrs/Nbs1) complex(Connelly 2002; Smith 1998). The ssDNA forms nucleofilament bounded by the DMC1 and RAD51 recombinases. Nucleofilaments search homologous chromosome as templates for repair, called strand invasion reviewed in Brown et al(2015). These inter-homolog strand invasion events are JM, precusor of dHJ. The other part of nucleofilament attaches this JM, finally producing dHJ(Schwacha et al., 1995). Previous research reported that the

resolution of dHJs is thought to result in COs, although NCOs are also theoretically possible(Allers et al., 2001). This pathway called Class I mechanism. COs can be devided into two categorized pathway, one is Type I also called ZMM pathway which involve in MSH4, MSH5, MER3, HEI10, ZIP4(Agarwal 2000; Chua 1998; Nakagawa 1999; Perry 2005; Storlazzi 1996). The remaining COs are Type II, MUS81 pathway that also required FANCM, RECQ4A, RECQ4B, MHF1, MHF2(Fabre 2002; Crismani 2012; Séguéla-Arnaud 2015; Girad 2014). NCOs are generted by an alternative pathway called SDSA, MMR (McMahill., 2007). In Arabidopsis thaliana, Spo11 topoisomerase produce DSBs(>200) over the chromsome, COs produced ~10, majority of ZMM pathway, and small fraction of COs undergoing Type II COs(<2), non interfering COs(Serrentino 2012; Mercier 2005).

The majority of crossovers are interfering that is resolved by ZMM pathway, meaning they are distributed further apart than expected poisson distribution. This uneven distribution is attributed to epigenetic factor. Recombination frequency show strong correlations with H3K4me3, H2A.Z, low nucleosome density(LND)(Choi et al., 2013). The first (+1) nucleosome downstream of TSS is highly positioned, contains the histone variant H2A.Z with H3K4me3(Choi et al., 2013). This region are gene – promoter region containing CTT repeat motifs along with H2A.Z which contribute to nucleosome exclusion, facilitate access of Spo11(Zhang et al., 2009). On the

other hands, crossover highly suppressed(called coldspots) by DNA methylation, transposons especially centromere(Choi et al., 2013).

The interference mechanism remains unclear, but a few models are explained that mechanical forces act over paired homolgous chromosomes(Zhang et al., 2014). Including interference mechanism, COs affected by natural genetic diversity such as level of sequence or structural polymorphism(Ziolkowski et al., 2017a). Also, juxtaposition of megabase homozygous and heterozygous regions in Arabidopsis is found to increase crossovers in the region of heterozygous(Ziolkowski et al., 2017b). Those are cis-acting factor controled recombination on that region. Along with cis-acting, there are also existed trans-acting regulation of recombination which acts on other region or the chromosome level. For example, genetic polymorphism explains the majority of crossover variation such as HEI 10 natural variation(Ziolkowski et al., 2017a). In addition to genetic variation, evidence also exists a effect on crossover by environmental stress especially temperature(Si et al., 2015).

There are still unsolved problem controling target region to break linkage drag that target region are co-segregate with undesirable gene. Also centromeres, highly methlyated region, are hard to isolate desirable gene cause of coldspot. Hyper-methylation regulation are expected to solve this problem and the approach of hyper-methylation achieve through manipulation of Type II mechanism,

non-interfering crossovers. Numerous reserach struggled to solve this problem and recently, in rice and arabidopsis, hyper-methylation achieved by recq4 mutant which induced COs to increase more than 3 fold change respectively(Mieulet 2018; Fernandes 2018). Hypo-methylation has a potential of reverse breeding and apomixis useful for assembling desirable segement and hybrid rice breeding. The strategy of clonal seed are also achieved by regulation of recombination related protein(Wang 2019; Fayos 2019).

SD is a phenomenon that observed genotypic frequencies of a locus fall outside the expected Mendelian segregation ratio(1:2:1). Several molecular mechanisms of SD and related gene have been clearly elucidated in rice(Harushima et al., 1996). S5 locus is a major factor of hybrid sterility leading to SD(Yang et al., 2012). Hybrid sterility is a major form of postzygotic reproductive barrier that restrict gene flow by killer-protector mechanism between inter-subspecific populations; japonica and indica. The S5 locus has three alleles, an indica allele S5-i, a japonica allele S5-j and a neutral allele S5-n(usually detected in wide-compatibility plant). The S5 locus has three tightly linkage gene, ORF3(protector), ORF4(partner), ORF5(killer). During female sporogenesis, the killer component of indica influence japonica result in premature programmed cell death and leads to SD(Yang et al., 2012).

Those SD effect emerge on mega-base(Mb) scale because of low rate of recombination frequency. For example, S5 locus SD phenomenon have been detected over 5Mb in previous research. S5 locus located on chromosome 6 upper arm that includes one of QTL cluster(Yamamoto et al., 2012). Also, Waxy gene and Hd3a gene that explain the majority of eating quality and heading date located in this region(He 1999; Monna 2002). The SD phenomen of S5 region makes it hard to use a desirable japonica allele.

Relationship among temperature stress, recombination frequency and SD have been reported in plants and microorganism(Si 2015; Lloyd 2018, Phillips 2015). Recombination are sensitive to temperature stress that recombination are exclusively changed by temperature stress from 3% up to 22%. Si(2015) reported that COs are changed approximately 22% through resequencing three samples. Hordium vulgare were rearranged chromosome genetic distance all over the chromosome by temperature stress(Phillips et al., 2015). In addition to rearrangement, recombination frequency were increasing in SD region of Hordium vulgare resulting from temperature. On the other hands, cold stress along with altitude had a effects on SD but cytoplasm had much stronger effect than altitude factor(Wnag et al., 2009).

Inter-subspecific breeding, one of the most basic and practical means of improvement of crop varieties, which assemble desirable allele between them based on homologous recombination. An

understanding of the patterns of segregation distortion and recombination is important for prediction of the phenotypic variation in the progenies. Previous research reported that temperature stress had a effects on recombination frequency by analyzing three to four samples(Si et al., 2015). When crossovers convert to recombination frequency by calculation of genetic distance(cM), generally use at least 100 samples per one populations. In this study, we used inter-varietal hybrid rice populations, DJ/NP, KS/NP, sampling at least 144 seeds per populations to represent the effects of temperature stress on recombination. Along with this, relationship between SD and temperature stress are largely unknown. We further studied relationship between SD and heat stress by KS/NP. This work would helpful for understanding of hybrid rice breeding to predict phenotypic variation.

MATERIALS AND METHODS

1. Plant materials and temperature treatment

Kasalath is a well known wide-compatibility Aus variety to rescue pollen viability(XIA et al., 2010). Nipponbare and Donjin are generally used japonica variety in experimental field bcause of T-DNA, Tos mutant line in Korea. DJ/NP, KS/NP F1 hybrid plants were grown in Suwon paddy field untile floral differentiation began to initiate. Plants were then transferred to a range of constant temperature, short day(12hr day at 30℃/ 12hr night at 25℃, humidity 70%) growth chambers to promote floral differentiation for 1 weeks. After then, temperature stress treated on each plants, DJ/NP 5 condition treatment : extreme heat (12hr day at 38℃ / 12hr night at 31℃), mild heat (12hr day at 34℃/ 12hr night at 28℃), control (12hr day at 30℃/ 12hr night at 25℃), mild cold (12hr day at 26℃/ 12hr night at 22℃), extreme cold(12hr day at 22℃/ 12hr night at 19℃) and KS/NP 2 condition treatment : Extreme heat and control for 3 weeks. As the duration of meiosis has previously reported 2 weeks(Moldenhauer et al., 2003), 3 weeks were chosen to be sufficienct to complete meiosis at each conditions without causing a significant impact on the differential period trajectory of the plants. We labeled the spikelet flowering within 10 days after temperature treatment finisihed to ensure fully temperature stress treatment. Then, harvested the seeds

from the labeled spikelet. Control condition set similar with Suwon average maximun / minimum temperature (30.6/23.0℃) to minimize temperature stress on control seeds(http://www.weather.go.kr/weather).

2. Pollen viability and seed fertility

Pollen sampling were similar with seed harvested condition, flowering within 10 days after temperature treatment finished. After sampling, pollens were stored in 70% EtOH. For each temperature condition, pollen viability was determined by Alexander staining(1% w/v) for three to four plants(Alexander, 1969). For each plant were counted at least 300 pollen grains. Also, seed fertility were counted over 300 seeds per each plant from the labeded spikelet for 3-4 replicates.

3. DNA extraction and genome wide SNP marker analysis

Genomic DNA was isolated from the seeds using the modified Phenol Chloroform Isoamlyalcohol (PCI) method.(Kamiya et al., 2003). Each DNA quality and concentration were determined using the Nanodrop spectrophotometer(Thermo Scientific, Wilmington, NC, USA)

On the basis of polymorphism in DNA sequences between Kasalath and Nipponbare genomes covering all 12 rice chromosome with average 3.3Mb interval, were developed in the Crop Molecular Breeding(CMB) Lab, Seoul National University (unpublished data).

We selected the Fluidigm assay based on three steps; first, collected a Fludigim assay previously developed in CMB. Second, in silico, check the target position that have polymorphic sequence from RiceVarMap v2.0(http://ricevarmap.ncpgr.cn/v2/). Thrid, pilot test are conducted to distinguish polymorphism of KS/NP. Finally we performed genotyping with 157 assays from 291 assays and determined recombination frequency using 116 assay(Table 1). SNP genotyping was conducted on Fludigim 96.96 Dynamic Array™ IFC & IFC controller and Fluidigm BioMark HD system (Fluidigm Corp, San Francisco, CA). Genotypes were determined using the Fluidigm SNP

Table 1. Fluidigm marker position and SNP type

Chr	Assay name			physical position (IRGSP 1.0)	Mb	NP	KS
1	id1000223	*		423620	0.4	T	G
1	cbm0103.4	*		3495000	3.5	A	C
1	id1004256			5333883	5.3	G	A
1	ad01003587	*		7436035	7.4	A	G
1	id1007185	*		9671547	9.7	A	G
1	ad01005318	*		11906192	11.9	T	G
1	id1009557			14431135	14.4	A	C
1	Os01g0371400	*	v	15290938	15.3	T	G
1	id1010652			17607448	17.6	T	G
1	ah01001478	*		20507838	20.5	A	G
1	SaF-CT			22376434	22.4	C	T
1	ah01001843	*	v	25183162	25.2	G	A
1	id1015984	*	v	27625670	27.6	C	T
1	id1018870	*		31307649	31.3	G	C
1	ad01015967			33249102	33.2	C	A

1	id1022407	*		35542178	35.5	G	A
1	qSH1-TG			36461792	36.5	T	G
1	SD1-GA			38384401	38.4	G	A
1	id1024836	*		39136724	39.1	C	G
1	P1193			40726147	40.7	G	T
1	ad01020824	*		41845080	41.8	A	T
2	id2000007	*	v	9619	0.0	T	C
2	ad02000512			2406171	2.4	T	C
2	id2002293	*	v	4361469	4.4	G	A
2	ah02000407	*	v	6710079	6.7	T	A
2	id2007512	*		19169633	19.2	T	T
2	ah02001499			21141852	21.1	T	G
2	id2009889	*	v	23977679	24.0	A	G
2	ad02011845			25961692	26.0	A	G
2	id2012773	*		29136459	29.1	T	T
2	ae02004877			30005577	30.0	G	C
2	cmb0232.7	*		31863984	31.9	A	G
2	cmb0235.4			34600940	34.6	G	A
2	id2016199	*		35312433	35.3	T	A
2	cmb0236.6			35770897	35.8	C	T
3	ad03000001	*	v	26505	0.0	C	A
3	id3000695			1107897	1.1	G	A
3	ah03000403	*	v	3525499	3.5	C	T
3	cmb0304.7	*	v	4731170	4.7	G	A
3	id3003462	*	v	5872068	5.9	A	G
3	cmb0306.9	*	v	6863874	6.9	T	A
3	ah03000736	*	v	7903738	7.9	G	A
3	cmb0308.8			8752021	8.8	C	A
3	id3005168	*	v	9975083	10.0	A	G
3	cmb0311.8	*	v	11798561	11.8	T	G
3	id3007541	*		15001183	15.0	A	G
3	GS3-CA			16733441	16.7	G	T
3	id3009433	*		19862435	19.9	T	C
3	id3010700	*		23494214	23.5	G	A
3	ad03013905			25037948	25.0	A	C
3	ad03014175	*	v	26324432	26.3	A	G
3	ae03006317	*		29888173	29.9	T	C
3	OS03g0733600(GIF1)	*	v	30048495	30.0	G	A
3	id3015453	*		32083416	32.1	A	G
3	ah03002520			34234299	34.2	C	T
3	cmb0336.5	*	v	35674217	35.7	A	G

4	P0610_1	*	v	228136	0.2	C	A
4	id4001096			2461396	2.5	T	A
4	id4002718			7008591	7.0	G	C
4	id4003524	*		11060807	11.1	T	G
4	id4004185			14137882	14.1	G	A
4	cmb0417.4	*		17276605	17.3	G	N
4	id4005704	*		19603606	19.6	A	T
4	cmb0420.7			20508025	20.5	G	A
4	cmb0422.7	*	v	22260801	22.3	G	T
4	ah04001252	*		28288983	28.3	A	G
4	id4009823	*		29515823	29.5	C	C
4	Os04g0615000(NAL1)	*	v	31212801	31.2	A	G
4	id4012434	*		35342951	35.3	T	T
5	xa5-TCAG			437500	0.4	TC	AG
5	cmb0501.9	*	v	1907766	1.9	A	T
5	qSW5-AG	*	v	5361396	5.4	A	G
5	id5004086	*		7999631	8.0	T	C
5	cmb0511.1			11098249	11.1	C	A
5	ah05000909	*		17364582	17.4	G	T
5	id5008218	*	v	19961728	20.0	G	T
5	ad05008445	*		21942245	21.9	C	T
5	cmb0529.7	*	v	29682002	29.7	C	A
6	id6000073	*	v	244274	0.2	G	A
6	GS6-TG			1465866	1.5	T	G
6	WAXY-TG			1765761	1.8	T	G
6	id6003373	*		4757948	4.8	A	A
6	S5-TC	*	v	5761511	5.8	T	C
6	cmb0607.0			7078662	7.1	A	C
6	cmb0607.8	*		7842653	7.8	T	T
6	id6005608			8726791	8.7	T	G
6	cmb0610.0			10085820	10.1	A	T
6	id6008118	*		13585906	13.6	T	A
6	cmb0614.6			14602162	14.6	G	A
6	Pid2-AG			17161572	17.2	A	G
6	cmb0618.2			17401069	17.4	C	T
6	id6011555	*		22259972	22.3	G	G
6	cmb0625.3	*		24487316	24.5	C	C
6	id6015530	*		27382490	27.4	T	C
6	cmb0629.3			28509971	28.5	A	T
6	id6016941	*	v	30809492	30.8	G	A
7	cmb0700.1	*	v	123298	0.1	T	A

7	ud7000187	*	v	2564992	2.6	A	G
7	cmb0703.2	*		3260078	3.3	G	C
7	ad07001853			4233777	4.2	G	A
7	id7001155	*		6987625	7.0	G	A
7	id7001998			11555965	11.6	A	T
7	id7002392			15007736	15.0	C	T
7	cmb0718.0	*		17356920	17.4	G	A
7	cmb0721.7	*	v	21048605	21.0	C	T
7	cmb0723.0	*		22354747	22.4	C	A
7	SLG7-GC			24666135	24.7	G	C
7	id7004645	*	v	25022403	25.0	C	T
7	cmb0727.0			26343254	26.3	T	C
7	cmb0728.5	*		27916720	27.9	T	C
7	cmb0730.3	*	v	29665190	29.7	C	T
8	cmb0801.3	*	v	1319679	1.3	G	T
8	cmb0802.8	*		2856476	2.9	C	T
8	wd8001250	*		8425664	8.4	T	C
8	id8006751	*		23652821	23.7	A	G
8	cmb0824.7			24711125	24.7	T	C
8	xa13-26013849-AG			26013772	26.0	A	G
8	GW8-AG	*		26502275	26.5	A	A
8	id8007764	*	v	27833564	27.8	G	T
9	id9000045	*	v	439538	0.4	T	C
9	id9000884			3670667	3.7	T	C
9	id9002419	*	v	7895936	7.9	G	G
9	cmb0909.6			8989303	9.0	T	A
9	id9003183	*		11848827	11.8	A	G
9	ae09005437	*		16803142	16.8	A	G
9	id9006953	*		19338953	19.3	A	C
9	TAC1-CT			20731844	20.7	C	T
9	id9007784	*	v	22600843	22.6	A	G
10	ud10000265	*	v	3983910	4.0	C	A
10	id10002069			6495219	6.5	G	T
10	id10002842	*		10660702	10.7	G	G
10	id10003706	*	v	14257303	14.3	T	C
10	wd10003790	*		19420303	19.4	C	A
10	ah10001182	*	v	21101395	21.1	C	A
11	id11000131	*	v	681662	0.7	G	A
11	cmb1102.6	*		2687952	2.7	T	T
11	cmb1107.1	*		7127396	7.1	A	T
11	wd11000649	*	v	9570096	9.6	A	G
11	cmb1109.8			9792348	9.8	A	G

11	id11004341			13098539	13.1	C	T
11	cmb1119.1	*		17019143	17.0	C	T
11	id11006897	*		19066989	19.1	T	C
11	Xa21-TC			21190035	21.2	T	C
11	cmb1127.4	*		25214331	25.2	G	A
11	id11011607	*	v	28896471	28.9	A	G
12	id12000076	*		265373	0.3	A	G
12	id12002113	*		4663607	4.7	C	T
12	cmb1207.0			7031031	7.0	C	T
12	id12003700	*		9127499	9.1	G	A
12	id12005212			14490400	14.5	T	C
12	id12006155	*		18315717	18.3	T	C
12	cmb1221.7			21612178	21.6	G	A
12	id12007742	*		22882888	22.9	T	G
12	cmb1224.0			23908519	23.9	A	G
12	cmb1226.0	*		25884498	25.9	C	T
12	id12010130	*	v	27434178	27.4	C	A

'*' indicated assay used for determining genotype

'v' means that we performed genotyping with two times to verify genotype data

4. Indel marker developments and genotyping

The DJ/NP polymorphic indel marker designed based on CMB Lab database(unpublished data)with by *in silico* approach (Primer3Plus software version 2; http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) (Table 2)

Indel marker genotyping was performed in total volume of 20ul containing 100ng of genomic DNA, 2μl 10X reaction buffer, 1μl dNTPs(10mM), 1μl each primer(10mM) and 1 Unit of Prime Taq polymerase(GENETBIO Co., Ltd., Korea). PCR was conducted using the following conditions : initial denaturation at 94℃ for 2 min,

followed by 35 cycles of denaturation at 94℃ for 30s, annealing at a range of experimental temperature(58 ~ 60℃) for 30s, and extension at 72℃ for 30s and a final extension at 72℃ for 10 min. The amplified PCR products were seperated by electrophoresiss on 2.5% ~ 3% agarose gel.

Table 2. DJ-NP polymorphic Indel markers on chromosome 1

Marker	Position	Forward	Reverse	Tm	Product size	
					NP	DJ
NPDJ0106	6693254	TGTCTTCCTCTTATGCTTGGATTGA	CCTGCAAGAAGAAAACCAGAAGAAT	60	164	173
NPDJ0107	7076857	CTCCGGTATATGTGGAGCCC	ACAGCTCGGATCATCAACGA	60	124	110
NPDJ0109	9425061	ACCATGTACGCAAGCGATCA	TTCTGAACACCTGCTGGACC	56	111	128
NPDJ0122.2	21763493	ACCGAGTTCGTACAGTATTTCACT	ATTCATGTTTCGACAAAGCTATCGG	56	236	210
NPDJ0128.6	28588024	GGTCTGACTGCATGCCTGAT	TTTTCCCCTTTCTGCACCCT	58	210	256
NPDJ0132	32130155	CCGTTCTGATAGCCACAGCA	GTGCTATACGCTGGCTGACA	58	176	202
NPDJ0136.2	36606184	AAAACTGTGGCACAAGTCAATAACT	TTTATAAATAGAACAAGCCCTCAGC	60	163	172
NPDJ0139.2	39394231	ATCCTCGTCTCTGCATGCTG	TGGTTGAGGGAATTCTCTCATCT	60	92	81

5. Data analysis

Genotype data were anylzed with iCIM mapping software(Li et al., 2008) to determined recombniation frequency and segregation distortion. Recombination was calculated using the Kosambi mapping function which incorporates crossover interference(Kosambi and Damondar, 2016). Segregation distortion was calculated using composition of single marker analysis and interval mapping. All Garph representations were performed in GraphPad Prism 8.2 (https://www.graphpad.com/scientific-software/prism/). All statistical anaylsis were done with R studio version 3.4.1(https://rstudio.com/).

6. Crossover counting

Allelic polymorphic data(NP, Hetero(H), KS) transduced to trinary variable(respectively, 0, 1 or 2) to count crossover events. We counted 2 events when changed from 0 to 2 and vice versa and 1 count when changed from 0 to 1 and 1 to 2 vice versa. No counting determined from no change of allelic variable. Crossover needs obligatory COs to provide physical tension for proper segregation. If COs events determined 0 by this method, it would be result from double COs or would not find properly. Together that, we exclude the data calling as a zero.

RESULTS

1. Pollen viability and seed fertility indicate stressful condition

During gametogenesis, gametes are vulnerable to temperature stress, pollen viability is an indicator of stressful effects on rice. NP/DJ populations only significantly decreased at 22℃ compared to control temperature(t-test, p=0.0002). Temperature of 26, 30℃ was not significantly changed compared to 30℃(t-test, p=0.0634, 0.2512 respectively). At 38℃, there were not observed matured pollens resulted in totally sterile. Japonica cultivar are susceptible to heat stress cause of adaptation in low temperature region rather than high temperature. This phenomenon was consistent with previously observed postmeiotic flowering defects when exposure to extreme high temperature(Shi et al., 2015). This results concluded that there were no temperature stress effects on pollen from 26℃ to 34℃. In KS/NP populations, pollen endured in the condition of extremely high temperature because Kasalath are Aus cultivar that indica species are tolerant to heat temperature. As a results, pollen viability of 38℃ were determined approximately 70% and significanlty decreased compared to control temeprature(t-test, p=0.0262). Seed fertility also show similar trends with pollen viability in both populations.

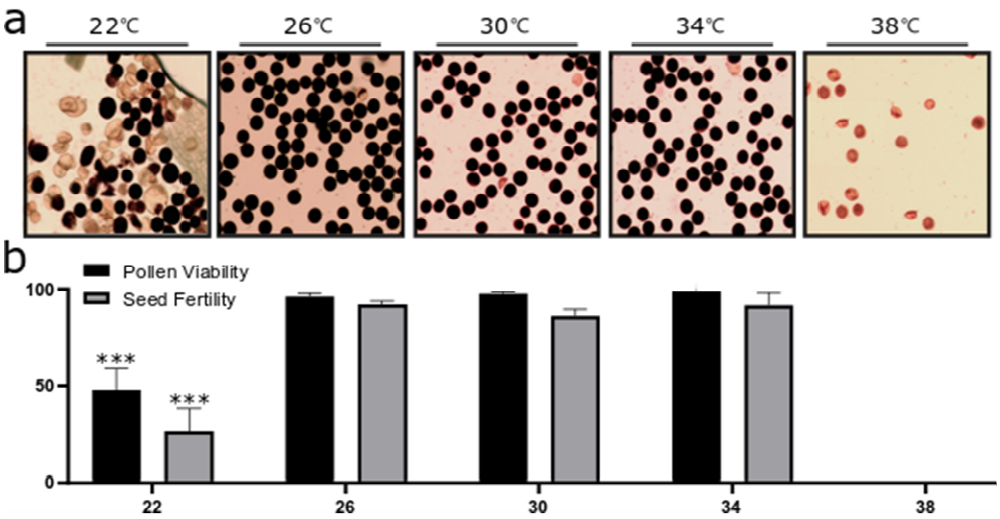


Figure 1. DJ / NP F1, pollen viability, seed fertility

(a) Pollen viability test with I-KI 1%(w/v) solution staining, (b) Seed fertility and pollen viability(t-test one-tail, *** $p<0.001$), the others t - test result in not significant(ns)

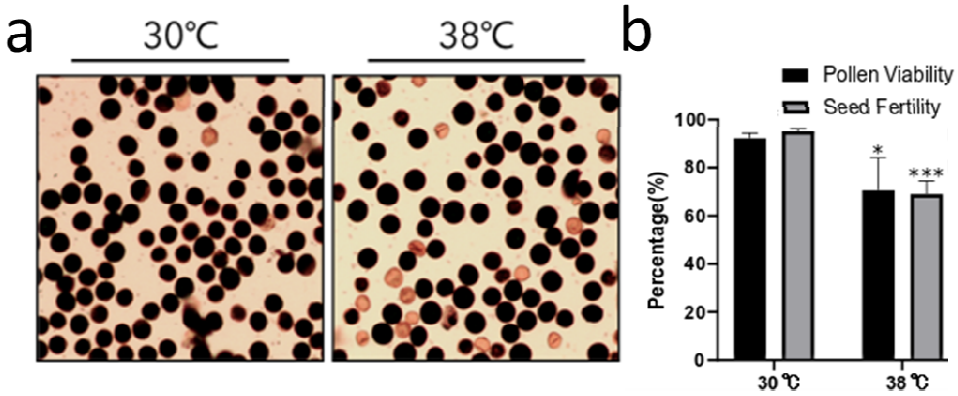


Figure 2. KS / NP F1 pollen viability, seed fertility

(a) Pollen viability test with I-KI 1%(w/v) solution staining, (b) Seed fertility

2. Crossover increase along with temperature on chromosome 1

Recombinaton frequency were overally increased in a range of experimental temperature (Figure 3), although there were not significantly increased on each region in both populations (wilcoxon signed rank test, $p > 0.05$). In NP/DJ populations, recombination decreased 4% compared to control temperature at 22℃ that pollen viability of NP/DJ was only significantly decreased. With a range from 26℃ to 34℃, there were not significantly change of pollen viability, the recombination frequency of 34℃ was increased 3% compared to control temperature. This results indicated that recombination frequency might be more sensitive in the condition of heat stress rather than cold stress. In KS/NP populations, recombination frequency was increased more than 11%. This results were similar to previously reported article that there were increasing trends that diminished at cold temperature and elevated at heat temperature. Also, calculated crossover number were sensitively changed at heat stess. Together that, recombination frequency might be more sensitively response to increase recombination frequency in heat stress and in sensitive in the conidition of cold stress that change within 5%.

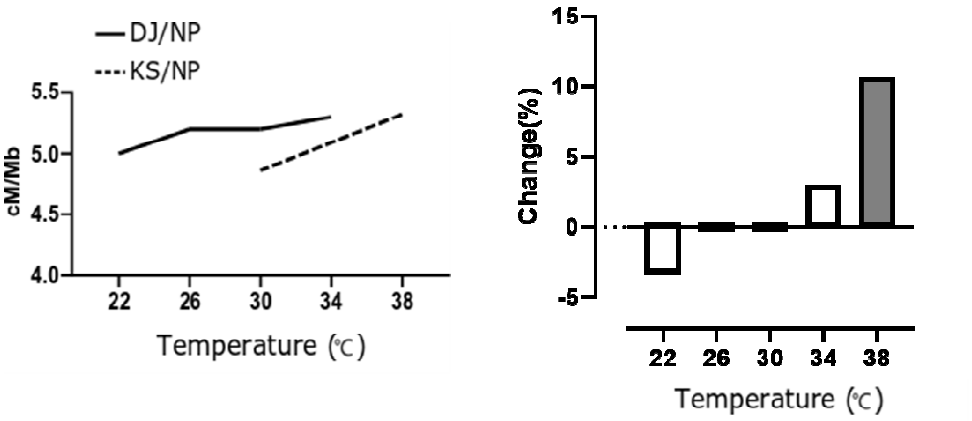


Figure 3. DJ / NP, KS / NP chromosome 1 recombination frequency

(a) DJ / NP, KS / NP recombination frequency. (b) DJ / NP, KS / NP change rate of recombination frequency

3. KS / NP fluigim genotyping results

Based on fluidigm data, we converted to genetic distance by iCIM mapping software(Figure 4). By combining physical length, genetic distance, and change rate, there were observed a strong correlation between physical length and genetic distance in control and heat stress condition(pearson correlation test, r=0.73 and p = 0.0065, r=0.83 and p = 0.0009, repectively), indicating that longer chromsome had more recombination events. However, there were not any positive correlation between change rate and physical length or change rate and genetic distance.(pearson correlation test, r = 0.39, p = 0.22 and r = 0.02, p = 0.96, respectively). Previous findings

reported that syneptonemal (SC) complex length, which was involved in ZMM pathway, had a positive correlation because they would be considered inducing factor of interference COs through protein-protein physical interaction. These results indicated that there might be not directed correlation between physical length and stress driven SC length change.

To determine which there were any change rate of recombination freqeuncy on each chromosomes, kmer clustering (k=4) was performed. Based on that, chromosome 2 are clustered the most increasing rate of change, 42.6%, from 3.96cMMb-1 to 5.62cMMb-1. Chromosome 1, 5,11 were denoted second cluster(average 14.57%, increase) and chromosome 3, 4, 7, 8, 10 were thrid cluster(average -0.9%, no change). The last cluster included chromosome 6, 9, 12 as a rate of change decrease(average -14.33%). This results were consistent with previous report that recombination change differ from each chromosome and this trends were not significantly different(Kolmogorov-sminrov test, p = 0.869). Together that, there would be unknown mechanism to rearrange recombination frequency on chromosome level when they exposed stressful condition (Table 3).

In KS/NP populations, COs number was determined by CO calculation methods that data was from fluidigm floursecence based genotyping data. COs number were average 24.5 per one samples that was somewhat differ from preivously reported since genotyping

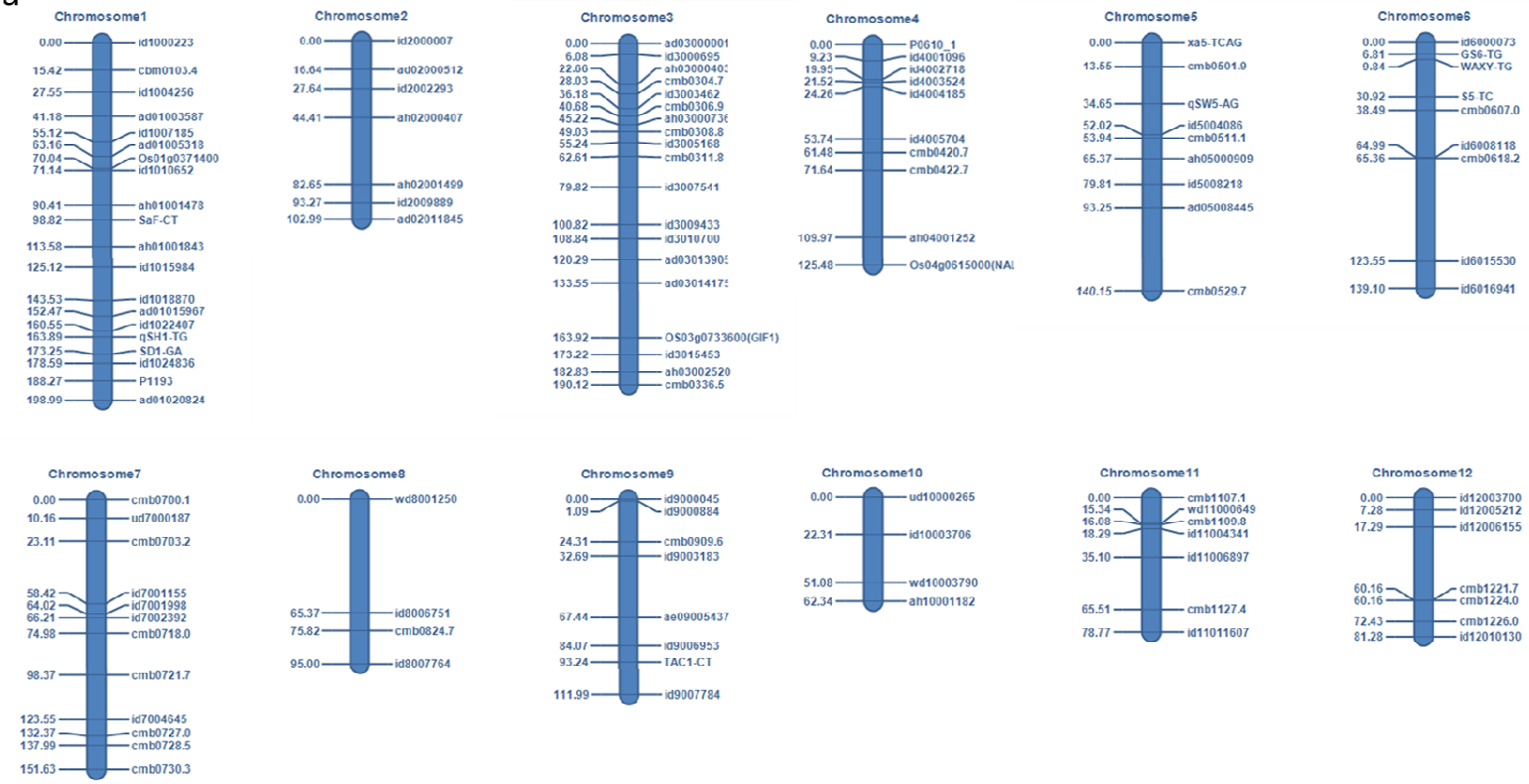
interval was too far to fully detect COs and double COs (Figure 5, a). Using t – test, we found that crossover rates were significantly increase at 38℃ compared to 30℃ with pollen viability decrease($p = 0.0388$). Despite of overall COs number were significantly change, we obtained that only chromosome 2 had a significantly change at 38℃(t-test, $p = 0.0019$) (Figure 5, b).

The landscape of recombination frequency along the chromosomes was found to be non-poisson distirbution. Using two sample Kolmogorov – Smirnov test on comparison of 30℃ and 38℃ recombniation frequency distribution, there were not significantly different on all chromosome (Figure 6, a). This results indicated that despite of recombination frequency increase, each region of all chromosome showed similar distribution. However, there were still distribution rearrangments, recombiantion frequency change in each region varied from -1 to 3 fold change (Figure 6, b)

COs in centromeres are highly suppressed by hyper DNA methylation. Megabase scale coldspot were located on centromeres and their pericentromeric regions. Except for chromosome 4, 7, all of these centromeres and pericentromeres were almost devoid of crossovers although chromosome overall recombination frequency increased more than 10% at 38℃. Chromosome 4 centromere region and pericentromere and chromosome 7 pericentromere elevated recombination frequency more than 2 fold change. Since centromeres and pericentromeres are coldspot that recombination suppressed

region, the change of chromosome 4 and 7 was clearly detected(from 0.60cMMb-1 to 3.52cMMb-1 and from 0.63cMMb-1 to 2.40cMMb-1, respectively). This trend recapitulates previous findings in Oryza sativa(tested along many stressful condition) of a increasing trend from 30℃ to 38℃.

a


Chromosome1
0.00 id1000223
15.42 cdm0103.4
27.55 id1004256
41.18 ad01003587
55.12 id1007185
63.16 ad01005318
70.04 Os01g0371400
71.14 id1010652
90.41 ah01001478
98.82 SaF-CT
113.58 ah01001843
125.12 id1015984
143.53 id1018870
152.47 ad01015967
160.55 id1022407
163.89 qSH1-TG
173.25 SD1-GA
178.59 id1024836
188.27 P1193
198.99 ad01020824
Chromosome2
0.00 id2000007
16.64 ad02000512
27.64 id2002293
44.41 ah02000407
82.65 ah02001499
93.27 id2009889
102.99 ad02011845
Chromosome3
0.00 ad03000001
6.08 id3000695
22.00 ah03000403
28.03 cmb0304.7
36.18 id3003462
40.68 cmb0306.9
45.22 ah03000736
49.03 cmb0308.8
55.24 id3005168
62.61 cmb0311.8
79.82 id3007541
100.82 id3009433
108.84 id3010700
120.29 ad03013905
133.55 ad03014175
163.92 OS03g0733600(GIF1)
173.22 id3015453
182.83 ah03002520
190.12 cmb0336.5
Chromosome4
0.00 P0610_1
9.23 id4001096
18.95 id4002718
21.52 id4003524
24.26 id4004185
53.74 id4005704
61.48 cmb0420.7
71.64 cmb0422.7
109.97 ah04001252
125.48 Os04g0615000(NAL
Chromosome5
0.00 xa5-TCAG
13.55 cmb0501.9
34.65 qSW5-AG
52.02 id5004086
53.94 cmb0511.1
65.37 ah05000609
79.81 id5008218
93.25 ad05008445
140.15 cmb0529.7
Chromosome6
0.00 id6000073
6.31 GS6-TG
9.84 WAXY-TG
30.92 S5-TC
38.49 cmb0607.0
64.99 id6008118
65.36 cmb0618.2
123.55 id6015530
139.10 id6016941
Chromosome7
0.00 cmb0700.1
10.16 id7000187
23.11 cmb0703.2
58.42 id7001155
64.02 id7001998
66.21 id7002392
74.98 cmb0718.0
98.37 cmb0721.7
123.55 id7004645
132.37 cmb0727.0
137.99 cmb0728.5
151.63 cmb0730.3
Chromosome8
0.00 wd8001250
65.37 id8006751
75.82 cmb0824.7
95.00 id8007764
Chromosome9
0.00 id9000045
1.09 id9000884
24.31 cmb0909.6
32.69 id9003183
67.44 ae09005437
84.07 id9006953
93.24 TAC1-CT
111.99 id9007784
Chromosome10
0.00 ud10000265
22.31 id10003706
51.08 wd10003790
62.34 ah10001182
Chromosome11
0.00 cmb1107.1
15.34 wd11000649
16.08 cmb1109.8
18.29 id11004341
35.10 id11006897
65.51 cmb1127.4
78.77 id11011607
Chromosome12
0.00 id12003700
7.28 id12005212
17.29 id12006155
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60.16 cmb1224.0
72.43 cmb1226.0
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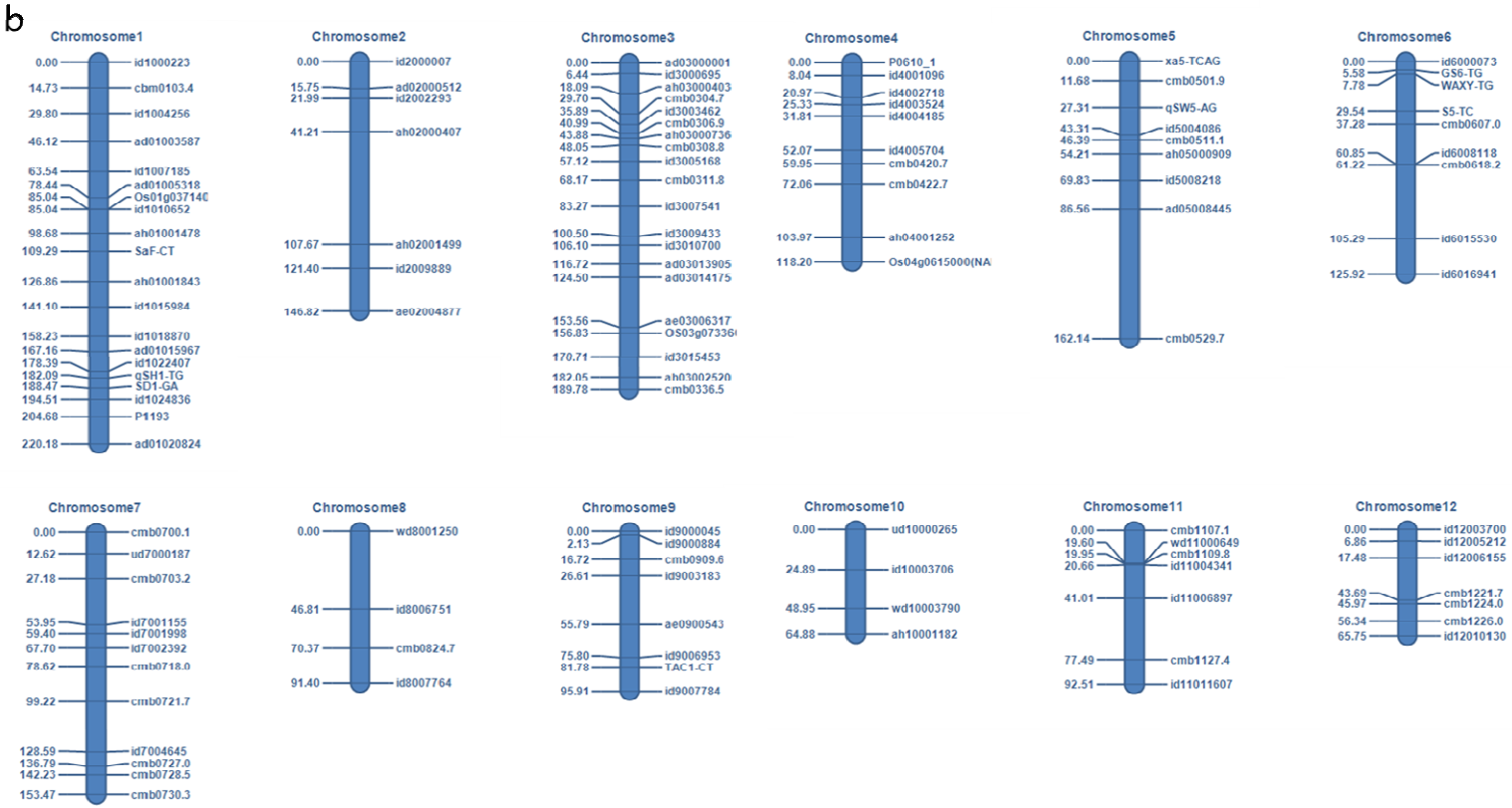

Figure 4. KS / NP iCIM mapping results.

(a) KS/NP 30℃ Left score indicated genetic distance(cM), right name indicated fluidigm. (b) KS/NP 38℃ iCIM mapping

Table 3 Crossover rates and rate of change on the rice chromosome

Chromosome	Physical length(Mb) / Coverage(%)	30℃			38℃			Change(%)
		Genetic distance(cM)	Recombination rate (cM/Mb)	CO No.	Genetic distance(cM)	Recombination rate (cM/Mb)	CO No.	
Chr1	43.3 (95.7)	198.99	4.81	3.8	220.18	5.32	4.0	10.6
Chr2	35.9 (74.1)	102.99	3.96	1.9	146.82	5.65	2.3	42.6
Chr3	36.4 (97.9)	190.12	5.33	3.5	189.78	5.32	3.6	-0.2
Chr4	35.5 (87.3)	125.48	4.05	2.3	118.2	3.81	2.2	-5.8
Chr5	30.0 (98.8)	140.15	4.73	2.3	162.14	5.48	2.4	15.7
Chr6	31.2 (98.0)	139.1	4.55	2.1	125.92	4.12	2.2	-9.5
Chr7	29.7 (99.4)	151.63	5.14	2.6	153.47	5.20	2.7	1.2
Chr8	28.4 (68.4)	95	4.90	1.5	91.4	4.71	1.6	-3.8
Chr9	23.0 (96.5)	111.99	5.04	2.1	95.91	4.32	1.9	-14.4
Chr10	23.2 (73.7)	62.34	3.65	1.4	64.88	3.79	1.4	4.1
Chr11	29.0 (75.0)	78.77	3.61	1.7	92.51	4.24	1.8	17.4
Chr12	27.5 (66.5)	81.28	4.44	1.6	65.75	3.59	1.5	-19.1
Total/Average	373.1/	1477.84/	/4.58	26.9/	1526.96/	/4.09	27.7/	/4.73

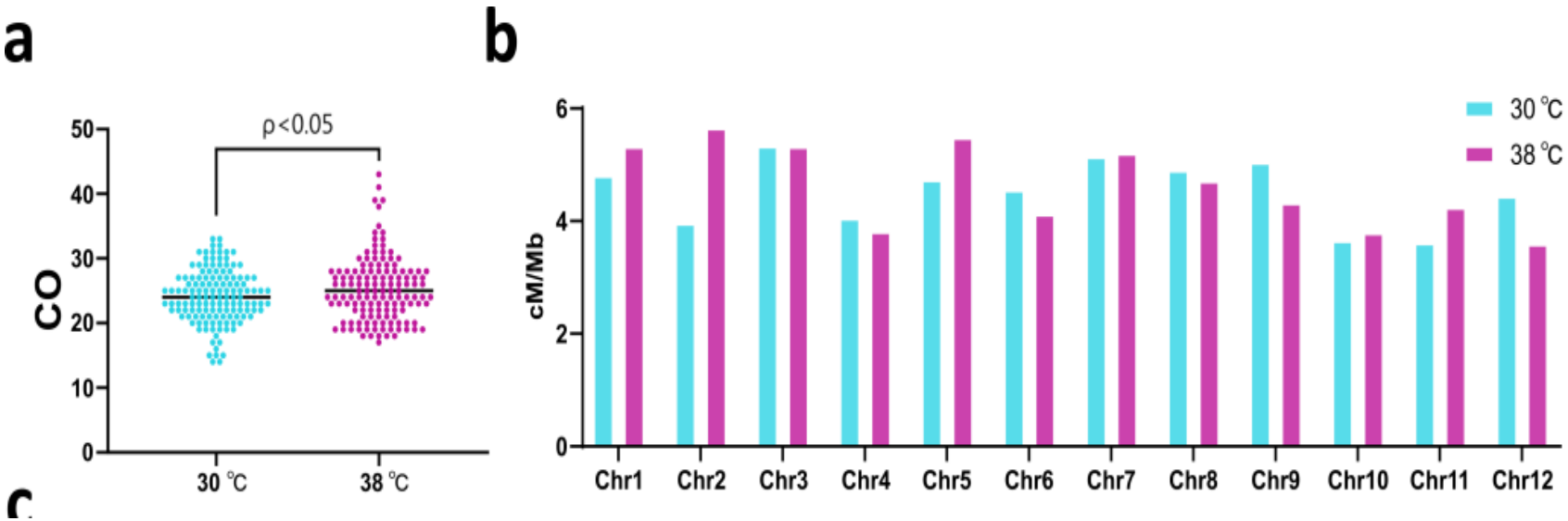

Figure 5. Results of KS / NP F_2 Crossover and genetic map size (a) Total COs per each F2 population. Tests are Mann-Whitney u test(p = 0.03, two-sided) on the calculated number of COs per F2 plants. (b) Genetic distance per physical map (cM / Mb) on each chromosome.

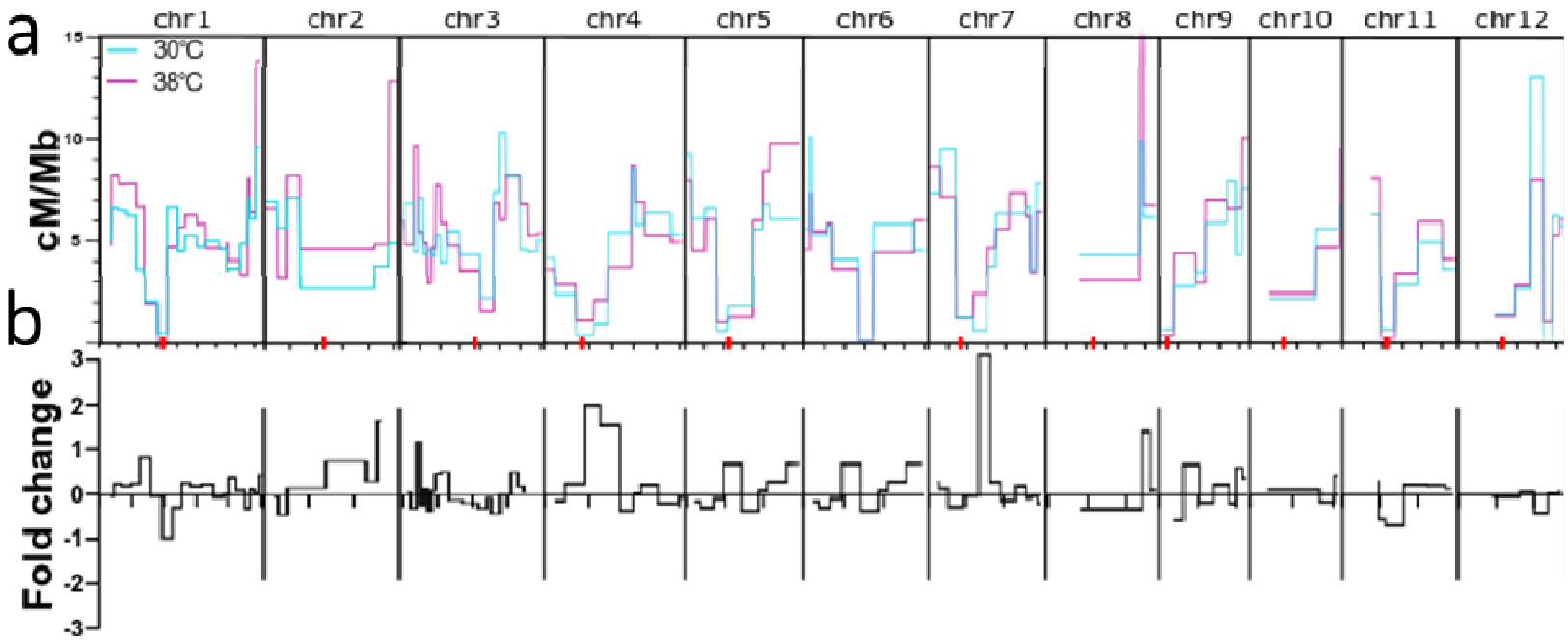

Figure 6. Results of KS / NP F_2 recombination landscape and frequency change (a) genome -wide recombination frequency landscape, red square indicated centromere region. (b) genome - wide recombination change rate, 38℃ recombination frequency / 30 ℃ recombination frequency

4. Segregation distortion was alleviated at heat stress ocndition

The chi-square test has been used to determine the segregation distortion , and we detected 6 region with 19 markers at 30℃ and 2 region with 3 markers at 38℃ (Table 4). There were two types of SD, KSSD and NPSD. Only one region, chromosome 4 was observed NPSD and the others were toward KSSD. The strong SD was observed in the chromosome 1, 3 which were LOD score 6.54, 5.11 respectively. Both of them were categorized as mSD based on previous research, there were sustained SD at 38℃. Chromosome 4, 6, 9, 10 were categorized as wheter fSD, cytoplasm effect, or unknown. Heat stress alleviated SD that all of them entirely avoid of SD except for the two mSD region. Chromosome 1, 3 which SD was sustained at 38℃, also diminish LOD score along with reduced SD region. Together that heat stress alleviated SD even if they maintained SD phenomenon. Previous findings reported that cytoplasmic effect are more influenced on SD rather than altitude(consistent with cold stress). However, temperature stress would be more important than cytoplasmic effects at high temperature.

To compare allele frequency of SD region, we selected the marker,peak of SD, in each chromosome and then tested frequency of 30℃ and 38℃ (Figure 7). One of five KSSD detected different

frequency and NPSD region was also significantly different(t-test, p = 0.0227 , p = 0.0066, respectively). The other regions of KSSD increased Nipponbare homo allele approximately 41% and decreased Kasalath homo allele about 14%. To verify allele distirbution in details, we tested Wilcoxon signed rank sum test(paired wilcoxon test). All region of KSSD(16 markers) significantly increased Nipponbare allele and decreased Kasalath allele(both of $p < 0.0001$). NPSD region are detected in chromosome 4(19.6Mb - 22.2Mb), all region changed significantly compared 30℃ with 38℃. In details, Kasalath homo allele increased about 2 fold change. In conclustion, high temperature stress was more influence than cytoplasmic effects and other sterility effects leading to SD, along with allele frequency rescued to Mendelian segregation ratio.

Table 4. Segregation distortion QTL locus

				30℃				38℃			
chr	Genetic Position(cM)	MarkerName	Type	LOD	Fitness(2)	Fitness(1)	Fitness(0)	LOD	Fitness(2)	Fitness(1)	Fitness(0)
1	90.41	ah01001478	mSD	4.9448	1	0.6091	0.2909				
1	98.82	SaF-CT		6.5464	1	0.4758	0.2742	3.3596	1	0.7755	0.3673
1	113.58	ah01001843		8.136	1	0.6727	0.1636				
1	125.12	id1015984		5.1127	1	0.6923	0.2692				
3	55.24	id3005168	mSD	3.4456	1	0.7604	0.3542				
3	62.61	cmb0311.8		5.1127	1	0.6923	0.2692	2.7089	1	0.5926	0.463
3	79.82	id3007541		4.4259	1	0.6204	0.3148	3.9168	1	0.6792	0.3396
3	100.82	id3009433		2.5188	1	0.8222	0.4222				
4	53.74	id4005704	unknown	2.5188	0.4222	0.8222	1				
4	61.48	cmb0420.7		3.9199	0.3333	0.8667	1				
4	71.64	cmb0422.7		3.3882	0.381	0.9524	1				
6	100.61	cmb0607.0	fSD	2.5164	1	0.6275	0.451				
6	108.19	S5-TC		3.3039	1	0.6667	0.3725				
6	129.27	WAXY-TG		4.1268	1	0.5818	0.3455				
6	132.3	GS6-TG		2.7434	1	0.566	0.4717				
9	67.44	ae09005437	cytoplasm	3.3039	1	0.6667	0.3725				
9	84.07	id9006953		4.604	1	0.5714	0.3214				
9	93.24	TAC1-CT		3.6858	1	0.6226	0.3585				
10	0	ud10000265	unknwon	2.7434	1	0.566	0.4717				

Fitness(2) : Fitness of marker type 2, estimated by size(2)/size(Max), Size(Max) be the maximum value of size(2), 0.5*size(2) and size(Max)

Fitness(1) : Fitness of marker type 1, estimated by size(1)/size(Max), Size(Max) be the maximum value of size(1), 0.5*size(1) and size(Max)

Fitness(0) : Fitness of marker type 0, estimated by size(0)/size(Max), Size(Max) be the maximum value of size(0), 0.5*size(0) and size(Max)

mSD' indicated male segregation distortion, 'fSD' indicated female segregation distortion

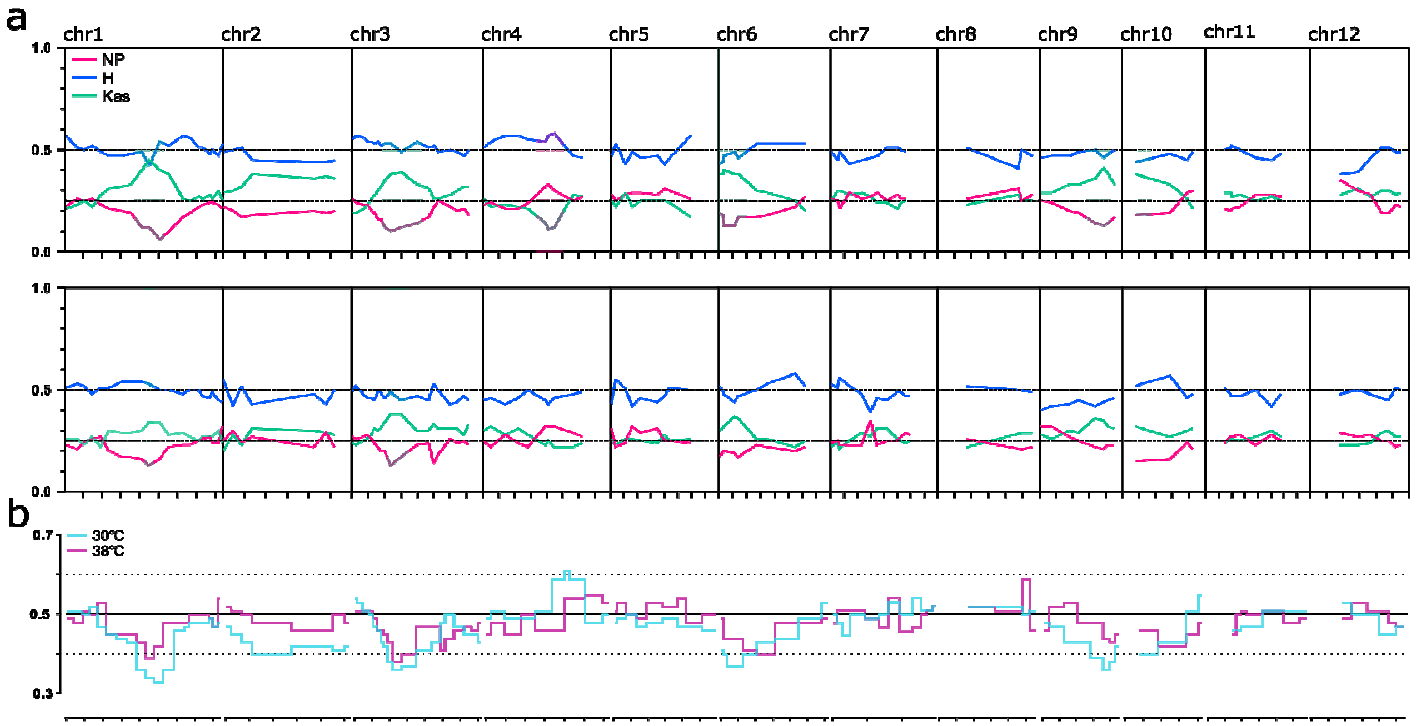

Figure 7. KS/NP whole genome segregation distortion analysis (a) 30℃ (above), 30℃ (below) allele frequency x-axis interval 5Mb, green boxplot indicate KS directed segregation distortion(KSSD), red boxplot indicate NP directed segregation distortion(NPSD). (b) NP allele portion, outside the range of dot line indicated segregation distortion.

5. Relationship among temperature stress, SD and recombination frequency

To examine the relationship among temperature stress, SD, and recombination frequency (Table 5), we tested Wilcoxon signed rank sum test and there were not significantly shift compared 30℃ with 38℃($p = 0.0625$). This results indicated that there were no obivous relationship between SD and recombination frequency. However, previous finding reported that recombination frequency were increased in the region of SD when exposure temperature stress. Together that, recombination frequency influenced on SD region, but not obivously important between recombination frequency and SD

Table 5. Recombination frequency in the region of segregation distortion

					30℃		38℃		
Chr	chromosome change (%)	Marker	Type	Physical position	cM	cM/Mb	cM	cM/Mb	change(%)
1	10.60	ah01001478	mSD	20,507,838	90.41	4.96	98.68	6.03	21.62
		SaF-CT		22,376,434	98.82		109.29		
		ah01001843		25,183,162	113.58		126.86		
3	-0.17	id3005168	mSD	9,975,083	55.24	4.89	57.12	5.20	6.39
		cmb0311.8		11,846,559	62.61		68.17		
		id3007541		15,001,183	79.82		83.27		
4	-5.80	id4005704	unknown	19,603,606	53.74	6.74	52.07	7.52	11.68
		cmb0420.7		20,508,025	61.48		59.95		
		cmb0422.7		22,260,801	71.64		72.06		
6	-9.40	GS6-TG	fSD	1,465,866	6.81	5.64	5.58	5.65	0.06
		WAXY-TG		1,765,761	9.84		7.78		
		S5-TC		5,761,511	30.92		29.54		
		cmb0607.0		7,078,662	38.49		37.28		
9	-14.30	ae09005437	cytoplasm	16,803,142	67.44	6.57	55.79	6.62	0.74
		id9006953		19,338,953	84.07		75.80		
		TAC1-CT		20,731,844	93.24		81.78		

DISCUSSION

COs are known as homeostatic control that regulate the emerging position leading to uneven distribution and obligatory COs to ensure proper segregation. CO numbers can be altered by intrinsic and extrinsic factors such as genetic factor, SNP and temperatures. Generally, there are so many findings in terms of relationship between temperature stress and recombination frequency. For example, barley(Hordium vulgare) were affected temperature stress and rearranged the recombination landscape. In arabidopsis, elucidated temperature stress mediated increasing recombination frequency was come from Type I pathway, ZMM pathway. By using resequencing of a few samples, recombination frequency increased as exposure of stressful condition. Although it is known that temperature stress can alter COs, there are no further studies which recombination landscape and specific region affected in under temperatures stress condition. Here, we demonstrated that in Oryza sativa, one of the major cultivated and early domesticated crop, these rearrangment of recombination frequency was formed through temperature stress and reconfirmed heat stress leads to elevate COs.

In here, we perform two inter-varietal populations of rice, DJ/NP, KS/NP. In DJ/NP, recombination frequency increased along with temperature stress increase. KS/NP were treated at 38℃ with significantly decrease of pollen viability result in increased

recombination frequency. As mention above, recombination frequency are largely observed that influenced on environmental stress, especially temperature stress. Recently studies indicated that some of other environmental stress condition did not alter recombination frequency. Together that, recombination frequency are clearly sensitive to temperature stress. In details, recombination frequency are largely detected increased at heat stress condition but varied in cold. Stress driven recombination increasing were correlated with SC length not physical length. Those are not same meaning that SC length were positively correlated with increasing recombination frequency, but physical length were not significantly correlated with change of recombination frequency. There are some nicely explained reasons why recombination frequency were sensitive to temperature stress. SC complex, the important role in which are communicate with other SC complex to induce physical tension(called as interference), displayed liquid crystal-like properties suggesting that there was a one possible mechanism, unique properties of temperature stress driven recombination elevation. Liquid crystal structures would be sensitively responsed by temperature stress and when exposed to heat stess condition, SC complexs are aggregates to form unusually axis organization and internal order leading to denaturation of SC complex. In rice previous research on relationship between environmental stress and recombination frequency, small portion of environmental stress were

increasing recombination frequency and only heat stress was significiantly increaseing among all heat treated samples. In our study, we also showed that heat stress are influenced on recombination frequency but not significantly at cold stress condition. Together that, crystal structure were sensitive to heat stress and somewhat insensitive to cold stress.

Our results has a similar increasing trends with other domesticated crops, Hordium vulgare, Oryza sativa, but not other species Arabidopsis thaliana, Drosophila melanogaster which was obeserved U-shape trends that recombination increased at both cold and heat temperature. The variation of recombination frequency that occurs under low or high temperature among species is a plastic response that is either adaptive in itself. Also, previous research reported that stress-driven recombination increasing mechanism would be different result from resolvase such as HEI10, MLH1 binding properties. It is also reconfirmed that cold driven recombination change is differ among speices by unknown mechanism, especially cultivated crops.

Not only chromosome overall size rearrangment, recombination landscape also differ compared 30℃ with 38℃. There are several methods for determining the recombiantion frequency. The prior method determined recombination frequency as calculation of genetic distance between two markers. Today, the advent of flourescent-tagged line(FTL) in arabidopsis, high through-put genotyping,

resequencing and powerful CO detection method, immunolocalization, there remains misinterpretation of relationship between environmental stress and recombination frequency especially by using FTL line. In here, we tested recombination frequency on each region. Despite of increasing recombination frequency, each region independently responed to temperature stress. This results also consistent with hordium vulgare analyzed by high through-put genotyping that each region was independently responed to temperature although recombination frequency were increased in broad level of chromosome. Also, recombination frequency also independently affected on chromosome. Together that, we concluded that to make clear suggestion, tested on at least whole genome level with low resolution.

The reason why recombination frequency differently responed to temperature stress, there were two potential explantaion for rearrangment and increase of recombination freuqnecy. First, recombination rearrangment go through DNA methylation rearrangment. Pollen DNA methylase upregulated to rearrange DNA methylation of pollen DNA as exposure of heat stress. Recombination frequency are started from DSBs and it was transcription factor-like properties, that binding the region of low DNA methylation, activation signal like H3K4me3, low nucleosome density region. It means that recombination precursor are rearranged by redistribution of DNA methylation. Second, protein structure properties leads to

recombination frequency rearrangment. Recently, sequence variation of HEI10 protein affects on recombination frequency. Similarly, when exposure heat stress, protein reshuffling by Heat-shock protein(Hsp), change the properties of protein. Also, as mention above, SC complex mediated interference mechanism, SC protein reshape that change the specific interference between protein-protein interaction.

SD restricts gene flow between indica-japonica hybrid rice leading disturbance of desirable allele uses. There are many previous research that induce SD such as structural variation mediated SD, killer-protector mechanism SD. There are categorized three types; mSD, fSD, cytoplasmic effect wheter they are affected on which type of reciprocal hybrid such as I/D//D, D//I/D. All type of SD were alleviated in heat stress condition. Based on previous research, cold temperature affected on SD and independently show avoidance or emergence along with temperature decrease. Together that SD are sensitively downregulated at heat stress but not at cold stress. There are possible explanation of this phenomenon. First, rearrangment of DNA methylation, as mention aboved, relocated on hybrid sterility gene promoter region to down regulation this effects. In chromosome 3, difference expression level of subspecific parents, leads to suppress the low expression parents(japonica type). Together that, we suggested that DNA methylation might be act as a key role in regulation of SD phenomenon at heat stress condition. Second, hybrid sterility caused by transposons and transposon methylation leads to

alleviate hybrid sterility along with SD. Indeed, numerous empirical studies have shown that hybrid genetic dysfunctions result from a broad variety of mechanisms related to maintenance of chromatin integrity. When exposure heat stress, transposons and other epigenetic instability inducing factor were downregulated by DNA methylation.

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초록

벼 품종에서 온도가 유전자 조환가에 미치는 영향

아종간 교배는 품종 개량에 있어 가장 기본적이고 실질적인 개선 수단으로 유전자 재조합에 기초하여 그들 사이에 바람직한 대립 유전자를 조립한다. 재조합과 함께 예기치 않은 표현형의 변이인 분리비 왜곡현상이 나탄난다. 재조합 빈도와 분리비 왜곡 현상의 이해는 자손의 표현형 변화를 예측하는데 있어 중요하다. 이전 연구결과들에 의하면 환경적 요인, 특히 온도가 재조합 빈도에 영향을 미치는 것으로 나타났다. 벼에서는 온도 스트레스와 유전자 재조환 빈도의 관계가 이미 보고되어있으나, 3 개에서 4 개의 표본을 사용하여 실험을 진행하였기에 이를 보완할 실험의 필요성이 있다. 그에 따라 우리는 최대 140 개 샘플을 사용하여 온도스트레스와 유전자 재조환의 관계에 대해 실험을 진행하였다. 결과에 따르면, 낮은 온도에서는 약 4% 유전자 조환가 빈도가 감소하고 높은 온도에서 최대 10% 증가하는 결과가 나타났다. 또한 고온에 노출될 때 아종간에 나타나는 분리비 왜곡현상이 완화되는 결과가 나타났다. 이 연구는 잡종 교배시 표현형 예측 변이를 이해하는데 도움이 될 것이라 기대된다.

주요어 : 벼, 온도 스트레스, 유전자 재조환, 교차, 분리비 왜곡, 고온 스트레스

학번 : 2018 - 23411

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