

Segregation distortion detected in six rice F₂ populations generated from reciprocal hybrids at three altitudes

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(Received 8 February 2009 and in revised form 9 June 2009)

Summary

This paper presents investigations of segregation distortion of six rice F₂ populations generated from reciprocal F₁ hybrids grown at three locations varied at altitudes from 400 to 2200 m. The F₁s were derived from reciprocal crosses between cv. XMG, which is a *japonica* landrace traditionally grown at 2650 m altitude, and cv. N34, which is a *japonica* restorer possessing a fertility restoring (*Rf*) gene and cytoplasm of male sterility (CMS) donated by an *indica* cultivar. Among nine morphological traits of the F₂ populations, only one was in normal distribution, eight were distorted in all or at least one population. Out of 16 polymorphic PCR markers, 10 markers distributed on 7 chromosomes were significantly distorted. Among these markers, RMAN7 and RM257 were distorted in both of the reciprocal populations, which suggested that nuclear genes had strong effects on segregation distortion. The other markers were distorted only in the populations with cytoplasm donated by XMG or N34. The results indicated that segregation of DNA markers was affected by cytoplasm background. Segregation distribution was also affected by altitude, since segregation distortions of most of the markers were detected not in all the three populations generated from F₁ grown at the three altitudes, but only in one population from F₁ grown at one altitude. Marker M45461, which is located within *Rf-1* locus, was severely distorted towards N34 in all the populations with cytoplasm donated by N34, but not in the populations with cytoplasm provided by XMG. The results indicated that interaction between CMS and *Rf* gene had strong effects on distortion. Results of this study indicated that *japonica* cytoplasm did not cause distortion favouring a special parent, but *indica* cytoplasm made distortion favouring a maternal parent. The results suggested that *indica* cytoplasm was not well compatible with *japonica* nuclear background, while *japonica* cytoplasm did not have such trouble with *indica* nuclei. This study also found that the six F₂ populations were divergent into two groups due to difference of cytoplasm background.

1. Introduction

Genetic distortion or segregation deviating from Mendelian ratios is not a rare case in segregated populations. Segregation distortion is the loss of specific genotypes in progeny, which was caused by gametic or zygotic selection due to a number of physiological or genetic factors, such as competition among gametes or pollens for preferential fertilization (Wendel *et al.*, 1987; Lyttle, 1991) and sexual-reproductive sterility due to inbreeding depression (Remington & O'Malley, 2000). It was correlated with increasing

genetic divergence between parental lines (Paterson *et al.*, 1991; Grandillo & Tanksley, 1996; Bradshaw *et al.*, 1998). Segregation distortion, which is considered to be a major genetic factor of divergence (McDaniel *et al.*, 2007), may favour genotypes of either parents or heterozygous alleles. Segregation distortion has been observed at morphological and molecular levels in the populations generated from heterozygotes derived from crosses between divergent parents in a wide range of organisms, such as in tomato (Rick, 1966), maize (Helentjaris *et al.*, 1986; Gardiner *et al.*, 1993; Lu *et al.*, 2002), pearl millet (Liu *et al.*, 1994), wheat (Manabe *et al.*, 1999), sunflower

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(Tang *et al.*, 2003), *brassica* (Saal & Struss, 2005), moss (McDaniel *et al.*, 2007) and *Arabidopsis* (Liu & Qu, 2008).

In rice (*Oryza sativa* L.), segregation distortion is also commonly detected in the populations descended from hybrids derived from crosses involving divergent genotypes (McCouch *et al.*, 1988; Sano, 1990; Cheng *et al.*, 1996; Xu *et al.*, 1997; Tan *et al.*, 1998; Liu *et al.*, 2001). The fundamental divergence in rice is divergence between *indica* and *japonica* subspecies (Chang, 1976), which is a result of ecogeographical differentiation associated with genetic adaptability to environmental conditions. *Indica* rice adapts to tropical and subtropical environment at low latitude and altitude areas, whereas *japonica* rice adapts to temperate environment at higher latitude or higher altitude areas (Wang *et al.*, 1992; Li & Rutger, 2000; Zeng *et al.*, 2001).

Analysis of segregation distortion based on F₂ populations derived from reciprocal F₁ hybrids grown at different altitudes will benefit to clarification of rice divergence caused by the effects of cytoplasm and altitude variation. In the present study, segregation distortion was investigated on morphological traits and molecular markers in six F₂ populations generated from hybrid zygotes grown at three locations varied at altitudes from 400 to 2200 m. The results indicated that, besides nuclear genetic factors, *indica* cytoplasmic background and cytoplasmic male sterility (CMS) strongly affected segregation distortion, and altitude variation also had effects on segregation distribution. Besides segregation distortion, the six F₂ populations were divergent into two groups according to their cytoplasm backgrounds.

2. Materials and methods

(i) Plant materials

Genetic stocks include two *O. sativa* cultivars, cv. XMG and cv. N34, F₁ hybrids derived from reciprocal crosses between XMG and N34 and six F₂ populations generated from the reciprocal F₁ hybrids grown at three locations, which are less than 200 km apart in direct distance, but varied at altitudes from 400 to 2200 m (Table 1). XMG is a *japonica* landrace traditionally grown in fields at 2650 m altitude; while N34, a restorer used in *japonica* hybrid rice, is an inbred line developed by multiple crosses involving *indica* and *japonica* cultivars, and it possesses cytoplasm donated by *indica* cultivar IR8 (Hong *et al.*, 2004; Tan *et al.*, 2004).

(ii) Collection and analysis of morphological data

Spikelet fertility (filled grains/total grains) and pollen fertility (fertile pollens/total pollens) were investigated

Table 1. Altitude and temperature of the three experiment locations

Site	YJ	KM	TJ
Altitude (m)	400	1860	2200
Latitude	23°34'	25°00'	25°05'
Longitude	102°09'	102°07'	102°36'
Annual average temperature (°C)	23.8	15.7	13.2
Annual maximum temperature (°C)	41.1	29.6	25.0
Average temperature at rice heading (°C)	26.6	19.2	16.5
Average maximum temperature at rice heading (°C)	35.5	25.3	21.6

with ten plants on two parents, and F₁ hybrids grown at three altitude sites. Anthers were collected from spikelets just before flowering, pollen grains were observed under a microscope after suspended in 1% (w/v) I₂-KI solution. Pollens in normal spherical shape and dark blue colour were regarded as fertile, pollens in irregular shape or without dark blue colour as sterile. Around 300 pollen grains from each sample were checked.

The two parents, two F₁s, and six F₂ populations were grown at 1860 m altitude (KM) in 2008. Nine morphological traits, including heading days, plant height, flag-leaf length, flag-leaf width, effective panicles, panicle length, panicle node length, total grains per panicle and spikelet fertility, were investigated on each progeny of six F₂ populations.

(iii) Genotyping by PCR markers

Total DNA was extracted from leaves of each sample with the Cetyltrimethylammonium Bromide (CTAB) method developed by Murray & Thompson (1980). The F₂ progenies were genotyped with 16 polymorphic PCR markers screened in previous research. PCR primers were synthesized by Takara Biotechnology (Dalian) Co., Ltd. according to the sequence by McCouch *et al.* (2002), Komori *et al.* (2004) and Jiang *et al.* (2006). Each PCR mixture had a final volume of 12.5 µl, containing 20–40 ng of genomic DNA, 0.3125 unit of Taq DNA polymerase (Takara Biotechnology), 100 µM each of dNTP mixture, 160 nM of primers and 1.25 µl PCR buffer (Mg²⁺ Plus). The PCR was performed on an Applied Biosystems 2720 Thermal Cycler Set with the following thermal program: initial denature at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 55 °C (adjusted according to annealing temperature of each primer) for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 7 min. The PCR products were separated by electrophoresis in 2.5% agarose

Table 2. Pollen and spikelet fertility of the reciprocal F_1 hybrids at the three altitudes

Altitude (m)	F_1 (XMG \times N34)		RF_1 (N34 \times XMG)		$F_1 - RF_1$	
	Pollen (%)	Spikelet (%)	Pollen (%)	Spikelet (%)	Pollen (%)	Spikelet (%)
400	92.20 \pm 2.42	89.89 \pm 3.97	50.19 \pm 4.98	81.29 \pm 6.41	42.01**	8.60**
1860	49.67 \pm 8.05	86.97 \pm 3.40	35.42 \pm 2.53	80.99 \pm 7.16	14.25**	5.98*
2200	46.98 \pm 6.48	86.21 \pm 4.54	28.62 \pm 3.38	78.88 \pm 7.12	18.36**	7.33*
F	171.3355	2.3675	85.61153	0.3693		
P	0.0000	0.1129	0.0000	0.6947		

* and ** represent significant levels of $P \leq 0.05$ and $P \leq 0.01$, respectively. It is the same in the followings table.

gel stained with ethidium bromide and photographed on an ultraviolet light-transmitting transilluminator.

(iv) Analysis of F_2 segregation

Normal distribution fitness of morphological traits of the F_2 populations was evaluated using Shapiro and Wilk's test (Shapiro & Wilk, 1965) with 7.05 version of DPS software (Tang, 2004; <http://www.chinadps.net/index.htm>). Segregation distortion of morphological traits was measured by skewness. PCR marker segregation ratios were tested by Chi-square using Microsoft Office Excel 2003. Genetic divergence among the F_2 populations was evaluated based on morphological traits and PCR markers. Genetic identities (I) and genetic distances (D) among the F_2 populations were calculated with Nei's method (1972) based on the data of PCR markers and ten graded morphological traits. Dendrogram and phylogenetic tree were constructed with the UPGMA method (Sokal & Michener, 1958) by using DPS 7.05, and PopGen32 (Yeh *et al.*, 1997) and TreeView (Page, 1996), respectively.

3. Results

(i) Effects of cytoplasm and altitude variation on fertility of reciprocal F_1 hybrids

The reciprocal F_1 hybrids had significant differences on pollen and spikelet fertility. The fertility of the F_1 s derived from XMG \times N34 was significantly higher than that of the F_1 s derived from N34 \times XMG at three altitudes. Especially, the difference in pollen fertility between the reciprocal hybrids was much higher (Table 2). The results suggested that cytoplasm had strong effects on fertility, especially on pollen fertility. Besides cytoplasm, altitude also affected fertility of the F_1 s. Pollen fertility of the F_1 s grown at low altitude was higher than that at high altitude. Pollen fertility of the reciprocal F_1 hybrids was as high as 92.20 and 50.19% at 400 m altitude, but it was only 46.98 and 28.62% at 2200 m altitude (Table 2). However, the

F_1 hybrids grown at different altitudes did not show statistical differences on spikelet fertility. The results indicated that altitude variation had significant effects on pollen fertility of the F_1 hybrids, but the effect was less obvious on spikelet fertility. The results suggested that altitude variation had selection pressure on pollen genotype, and such fertility difference could lead to genotype selection of descendant progenies.

(ii) Effects of cytoplasm and altitude variation on segregation distortion of morphological traits

Among nine morphological traits, only flag-leaf length fitted in the expected normal distribution in all six F_2 populations (Fig. 1*i*), the other eight traits were significantly ($P < 0.05$ or $P < 0.01$) deviated from normal distribution in almost all F_2 populations. Three traits, effective panicles, panicle length and spikelet fertility, were significantly distorted toward N34 in all the populations (Fig. 1*a-c*), while flag-leaf width and panicle node length were significantly skewed toward XMG (Fig. 1*d,e*). All of those five traits showed severe distortion. The other three traits, total grains per panicle, plant height and heading days, were distorted toward different parents in different populations with low skewness values (Fig. 1*f-h*).

The F_2 populations generated at different altitudes showed much different segregation distortions. Flag-leaf width had the largest skewness towards XMG in the two populations generated at 400 m altitude, while it showed smaller distortion in the populations generated at other locations (Fig. 1*d*). Heading days showed the largest skewness in the populations generated at 2200 m altitude (Fig. 1*h*), and panicle length and panicle node length showed the largest distortion in the populations generated at 1860 m altitude (Fig. 1*b,e*).

Different traits showed different segregation distortions on different cytoplasm. For example, skewness values of spikelet fertility were larger in the populations with XMG cytoplasm than those in the populations with N34 cytoplasm (Fig. 1*c*), while

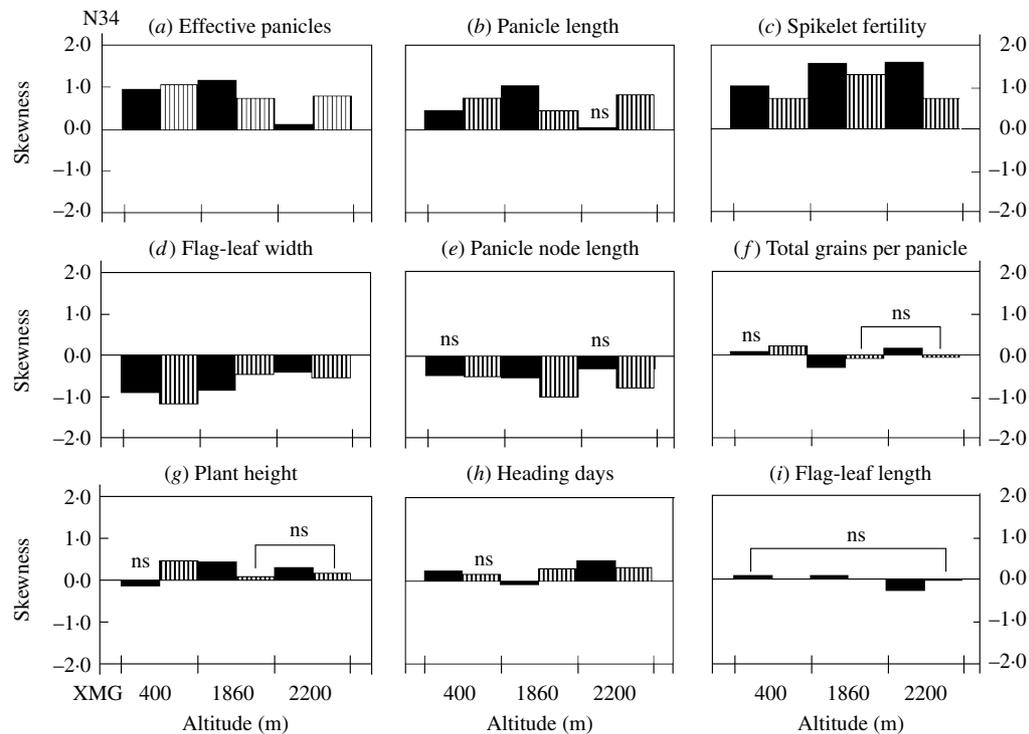


Fig. 1. Skewness of morphological traits of the reciprocal F_2 populations generated at the three altitudes. Skewness values of the F_2 populations are represented by column height, in which black and line ones represent the populations with cytoplasm from XMG and N34, respectively. The 'ns' indicates the distortion is not statistically significant.

skewness values of panicle node length were larger in the populations with N34 cytoplasm (Fig. 1e).

Besides, segregation distributions were also affected by interaction effects of cytoplasm and altitude variation. For examples, skewness value of panicle length was larger in the population with XMG cytoplasm generated at 1860 m altitude, but they were larger in the populations with N34 cytoplasm at other altitudes (Fig. 1b). Interaction effects of cytoplasm and altitude were also observed on other traits, such as effective panicle (Fig. 1a).

(iii) Segregation distortion of DNA markers

Segregation distortion of the F_2 populations was also detected by DNA markers. Out of 16 loci, only six markers were in the Mendelian ratios, and 10 markers distributed on 7 chromosomes were significantly distorted from the expected 1 : 2 : 1 or 3 : 1 ratio ($P < 0.05$ or $P < 0.01$). Among them, six markers, which were located on chromosomes 2, 8, 9, 10 and 11, skewed toward N34, and three markers, which were located on chromosomes 3 and 5, skewed toward XMG; only RM218, which was located on chromosome 3, skewed favouring heterozygote (Table 3). Seven markers showed distortion in the populations generated at 400 m altitude; four and five markers showed distortion in the populations generated at 1860 and 2200 m altitudes, respectively.

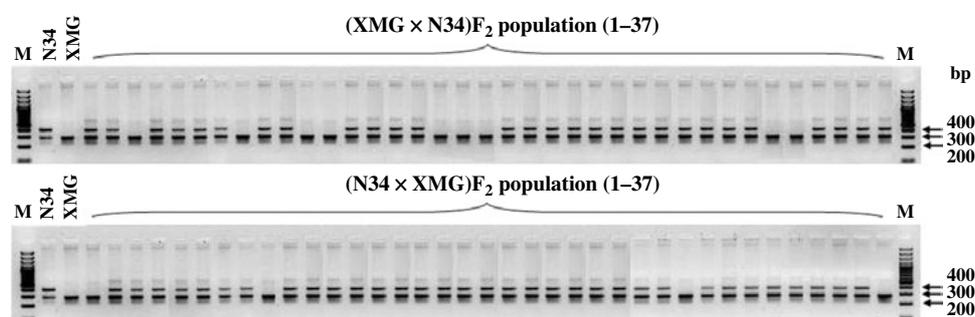
Only RMAN7 and RM257, which were located on chromosomes 2 and 9, respectively, were distorted in both of the reciprocal F_2 populations, favouring N34, while most of markers were distorted just in one of the reciprocal populations, possessing cytoplasm of N34 or XMG. Furthermore, all the markers that showed distortion in the populations with N34 cytoplasm were skewed toward N34, while the markers that were distorted in the populations possessing XMG cytoplasm did not show the same favouring type, three favouring XMG, two favouring N34, one favouring heterozygote. The results indicated that cytoplasm from N34 had stronger effects than that from XMG on the segregation, especially for loci of RM333 and M45461. Marker M45461, a dominant marker (Fig. 2) located on chromosome 10, was extremely distorted towards N34 in the populations possessing N34 cytoplasm generated at the three altitudes, but it fitted in Mendelian ratio in the populations possessing XMG cytoplasm. Marker RM333 showed a similar segregation model (Table 3).

(iv) Divergence of the F_2 populations

Both dendrogram on morphological traits and phylogenetic trees on PCR markers showed that the F_2 populations were divergent according to the cytoplasm and the altitude variation. The six F_2 populations were divergent into two groups (Fig. 3).

Table 3. Marker segregation ratios of the F_2 populations

Marker	Chromosome	F_2 -generated altitude (m)	(XMG \times N34) F_2					(N34 \times XMG) F_2				
			Population sizes	XMG	H ^a	N34	χ^2	Population sizes	XMG	H ^a	N34	χ^2
RMAN7	2	400	175	1	2	1	1.65	131	1	2.2	1.7*	6.24
		1860	185	1	2	1	4.82	182	1	2	1	4.67
		2200	181	1	1.9	1.6**	10.39	186	1	2	1	3.85
RM218	3	400	175	1	2	1	0.79	131	1	2	1	1.6
		1860	185	1	2	1	1.57	182	1	2	1	0.1
		2200	181	1	3.4**	1	12.22	186	1	2	1	1.74
RM16	3	400	175	1.7*	2.4	1	7.05	131	1	2	1	1.85
		1860	185	1	2	1	1.44	182	1	2	1	2.35
		2200	181	1	2	1	2.17	186	1	2	1	1.47
RM545	3	400	175	1.3*	1.6	1	6.45	131	1	2	1	0.45
		1860	185	1	2	1	2.3	182	1	2	1	0.8
		2200	181	1	2	1	3.85	186	1	2	1	2.09
RM440	5	400	175	1	2	1	0.1	131	1	2	1	1.92
		1860	185	1.5*	2.0	1	6.28	182	1	2	1	1.68
		2200	181	1	2	1	4.13	186	1	2	1	0.41
RM152	8	400	175	1	2	1	2.07	131	1	2	1	1.12
		1860	185	1	2	1	3.55	182	1	2	1	4.93
		2200	181	1	2	1	1.87	186	1	1.9	1.5**	9.90
RM257	9	400	175	1	1.9	2.2**	31.73	131	1	6	6.1**	40.63
		1860	185	1	2.6	1.9**	9.53	182	1	1.5	1.1*	6.16
		2200	181	1	2	1	5.32	186	1	2	1	2.77
RM333	10	400	175	1	2	1	0.15	131	1	4.3	2.9**	14.86
		1860	185	1	2	1	1.09	182	1	4.0	5.1**	68.11
		2200	181	1	2	1	0.31	186	1	4.3	4.1**	41.39
M45461 ^b	10	400	175	1		3	0.05	131	1		12.1**	21.07
		1860	185	1		3	0.65	182	1		35.4**	48.07
		2200	181	1		3	0.05	186	1		61.0**	54.26
RM209	11	400	175	1	2	1	4.82	131	1	2.0	1.6*	7.15
		1860	185	1	2	1	0.66	182	1	2	1	3.88
		2200	181	1	2	1	0.98	186	1	2	1	1.39

^a Heterozygote.^b A dominant marker.Fig. 2. Segregation of dominant marker M45461 in the reciprocal F_2 populations generated at 1860 m altitude.

One group consisted of the three populations generated from the same hybrids of XMG \times N34 grown at different altitudes, which possessed cytoplasm from XMG. Another group consisted of the three

populations derived from the reciprocal cross, which possessed cytoplasm donated by N34. In both groups, the genetic divergences between the populations generated at high altitude (2200 m) and low altitude

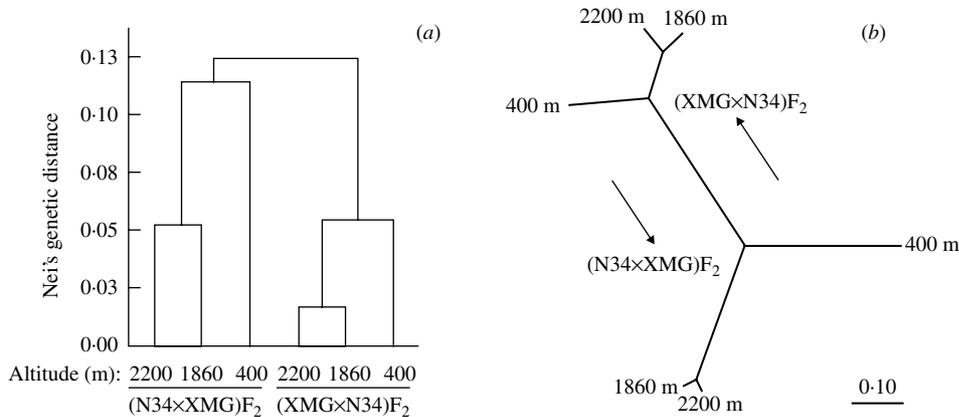


Fig. 3. Dendrogram (a) and phylogenetic tree (b) of the six F₂ populations based on the Nei's genetic distances on morphological traits and molecular markers, respectively.

(400 m) were larger than those between middle altitude (1860 m) and high altitude or between middle altitude and low altitude.

4. Discussion

Segregation distortion, which is a common case in segregated populations derived from crosses between divergent parents, can be caused by a number of physiological or nuclear genetic factors (Matsushita *et al.*, 2003; Liu *et al.*, 2008). This study found that the effects of nuclear genes, cytoplasmic genes and altitude variation could cause segregation distortion. For instance, distortion of markers RMAN7 and RM257 was caused by nuclear genetic factors, since these two markers were distorted in both of the reciprocal populations, regardless of cytoplasm, especially for RM257, which was distorted in both of the reciprocal populations generated at the same altitudes. Nuclear genetic factors involved in segregation distortion, such as gametophytic selection gene, were also reported in other studies (Wilson and Levin, 1986; Matsushita *et al.*, 2003; McDaniel *et al.*, 2007).

Although nuclear factors played a key role for segregation distortion, which was reported in a lot of studies (Bradshaw *et al.*, 1998; He *et al.*, 2001; Goloenko *et al.*, 2002; Jiang *et al.*, 2006; Liang *et al.*, 2007; Liu *et al.*, 2008), results of this study indicated that cytoplasm and altitude variation also had effects on distortion of morphological traits, although effects of dominant genes could not be excluded. Segregation distortions related to effects of cytoplasm were also reported in other studies (Faris *et al.*, 1998; Manabe *et al.*, 1999; Werlemark *et al.*, 1999; Goloenko *et al.*, 2002).

Among the ten markers that showed segregation distortion, marker M45461, which was located within fertility restoring gene *Rf-1* locus (Komori *et al.*,

2004), showed extreme distortion in the F₂ populations possessing N34 cytoplasm, but its segregation fitted Mendelian ratio in the reciprocal populations possessing XMG cytoplasm. A reasonable cause for that is N34 possesses a fertility restoring gene, *Rf-dl* gene, which is allelic to *Rf-1* (Hong *et al.*, 2004; Tan *et al.*, 2004) and CMS (Wang *et al.*, 2009), but XMG possesses normal cytoplasm. Therefore, it is expected that segregation of M45461 favoured N34 in the F₂ populations on CMS cytoplasm. By contrast, it is also expected that segregation of this marker in the reciprocal F₂ populations fitting Mendelian ratio, since the progenies did not possess CMS. Marker RM333, which is about 12.8 cM from M45461 (<http://blast.ncbi.nlm.nih.gov/Blast.cgi> and <http://www.gramene.org>), also showed similar distortion. Severe segregation distortion of these two markers could be caused by male gamete selection favouring the male gametes possessing *Rf* genotype due to interaction between CMS and *Rf* gene. Significant difference on pollen fertility between the reciprocal F₁ hybrids was an evidence of the male gamete selection due to existence of CMS. Segregation differences of these two markers between the reciprocal F₂ populations were a good example that interaction between cytoplasm and nuclear gene caused segregation distortion due to male gamete selection.

Besides CMS, cytoplasmic background also affected segregation distortion as reported in other studies (Faris *et al.*, 1998; Manabe *et al.*, 1999; Werlemark *et al.*, 1999; Goloenko *et al.*, 2002). This study found that segregation distortions of the markers did not favour a special parent in the F₂ populations possessing cytoplasm from XMG, but the distortions in the F₂ populations possessing N34 cytoplasm favoured cytoplasm donor N34. A difference between the two cytoplasm is that cytoplasm possessed by N34 was from an *indica* cultivar through multiple crosses involved in *indica* and *japonica* cultivars (Hong *et al.*,

2004), while the cytoplasm possessed by XMG is from a *japonica* landrace grown at fields with 2650 m altitude. The results are consistent with observation that distortions in the segregated populations possess *indica* cytoplasm favoured *indica* parents (He *et al.*, 2001; Peng *et al.*, 2006; Liang *et al.*, 2007). These results indicated that *japonica* cytoplasm did not cause distortion favouring a special parent, but *indica* cytoplasm resulted in distortion favouring a maternal parent. The results also suggested that *indica* cytoplasm was not well compatible with *japonica* nuclear background, but *japonica* cytoplasm did not have such a problem with *indica* nuclei. According to such clues, *japonica* cultivars should be used as maternal parents in crosses between *indica* and *japonica* cultivars for developing *japonica* varieties.

Besides cytoplasm sources, results of this study also revealed that altitude variation had effects on segregation distortion. Segregation differences between the populations generated at different altitudes could be caused by the temperature differences among the three locations, which is an obvious difference of the environmental conditions among the locations. Segregation ratio affected by environmental conditions was also reported in *Phytophthora sojae* (MacGregor *et al.*, 2002), and temperature stress resulting in segregation distortions were observed in other organisms, such as in *Drosophila melanogaster* (Mange, 1968; Hiraizumi, 1993) and tomato (Zamir *et al.*, 1982).

This study revealed that the six populations were divergent into two groups on two cytoplasm, and then the groups were further differentiated according to similarity of altitudes where the populations were generated. The divergence could be resulted by difference of distortions among the populations, since distortion is a reflection of divergence (Tanksley & Nelson, 1996). The results indicated that cytoplasm and altitude had effects on divergent selection, which was a major factor for crop evolution (Michelmore & Meyers, 1998), but cytoplasm had much stronger effects than altitude factor.

This work was supported by National Natural Science Foundation of China (No. 30860065) and by Yunnan Provincial Science and Technology Department, China (2006NG05).

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