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Subspecies characteristics in filial generation of cross between indica and japonica rice under different environments

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Abstract The recombinant inbred lines (RILs) population derived from the cross between “Zhongyouzao8” (hsien or indica) and “Fengjin” (keng or japonica) was grown in Liaoning and Sichuan Provinces to compare the subspecies characteristics under different environments. The results showed that the distribution of Cheng’s index was japonicalinuous. The population was more japonicalinuous in Sichuan than that in Liaoning as a whole. Further analysis indicated that indica and indica-cline in Sichuan were more indicalinuous than those in Liaoning, japonica and japonica-cline in Sichuan were more japonicalinuous than those in Liaoning. The effects of environment on six characteristics of Cheng’s index were different because of different characters, as well as on the correlations between the six characters, the Cheng’s index and the vascular bundle traits. For example, the length of the first and second rachis and leaf pubescence were more japonicalinuous in Sichuan than in Liaoning, while the length/width ratio of spikelets and glume color at heading time were more indicalinuous in Sichuan than in Liaoning. The vascular bundle characteristics of RILs in different environments changed remarkably. The number of large vascular bundles in panicle neck was less in Sichuan than in Liaoning, while the number of small vascular bundles in panicle neck and second top stem was greater in Sichuan than in Liaoning. The ratio of large to small vascular bundles in panicle neck decreased and the ratio of large vascular bundles between the second top stem and panicle neck increased in Sichuan. Though total trend was the vascular bundle ratio of large to small in indica

was greater than that in japonica and the ratio of large vascular bundles in indica was less than that in japonica, it is not appropriate that the vascular bundle ratio of large to small and the ratio of large vascular bundles are regarded as the parameters for subspecies classification in rice. The vascular bundles characteristics from typical indica or japonica were recombined in the population derived from indica and japonica crossing.

Keywords rice, environment, cross between indica and japonica rice, subspecies classification of indica and japonica rice

1 Introduction

The cultivated Asian rice species is composed of two subspecies, indica and japonica. In China, indica and japonica cultivation are equally important. Indica and japonica were known by our ancestors two thousand years ago. During a long history, the plantation patterns that indica and japonica were cultivated in south China and in north China, respectively, have come into being (The Editorial Board of Ding Ying Rice Symposium, 1983; Cheng, 1993; Zhang and Xu, 1998). Indica and japonica are very apparently different in biological characteristics, with their advantages and disadvantages respectively in rice production (Yang et al., 1962; Tsunoda and Takahashi, 1984; Cheng, 1993). Ding (The Editorial Board of Ding Ying Rice Symposium, 1983) considered that indica and japonica belonged to different climate-ecology types; indica was a basic type in cultivars and japonica was the variation type that came from the indica differentiation from low altitude and low latitude region to high altitude and high latitude regions. Great heterosis and variations can be created from crossing indica and japonica (Yang and Zhao, 1959; Zhu et al., 1964; Chen et al., 1995a; Yuan, 1997; Sun et al., 2000). The intersubspecies heterosis utilization has become a hot point in rice breeding either in northern or southern China (Yang et al., 1962). Investigations based on rice pedigree indicate that the most cultivars widely used in

Translated from *Acta Agronomica Sinica*, 2007, 33(3): 370–377 [译自: 作物学报]

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northern China were bred through the cross between indica and japonica, as well as in southern China (Lin and Min, 1991). When the vascular bundles (Xu et al., 1997; 1998; 2003a; 2003b), Cheng's index (Cheng et al., 1984; Xu et al., 2003b) and stomata in leaves (Chen et al., 1995b) were studied, the results showed that cultivars derived from the cross between indica and japonica in northern China did not combine the subspecies characteristics; in contrast, they retained the japonica characteristics. It suggested that the results might be correlated with ecological adaptability or reproductive isolation without conscious direct selection (Xu et al., 2003b). Up to now, few reports are available on the subspecies characteristics changes in filial generations of the cross between indica and japonica under different environments. This paper is aimed at understanding these facts.

2 Materials and methods

Material used in this study was F_6 (RILs) derived from the cross between "Zhongyouzao8" (indica) and "Fengjin" (japonica), with 176 lines, each of which was divided into two (short for RILSA), numbered on lines, and grown in Deyang in Sichuan Province (N 30°31'–31°42', E 103°45'–105°15') and Shenyang in Liaoning Province (N 41°11'–43°2', E 122°25'–123°48'), respectively. In Sichuan, the materials were grown by 20 plants per line, sowed on April 26, and transplanted on May 27, with 26.6 cm row spacing and 16.5 cm plant spacing. In Liaoning, the same materials were grown by ten plants per line, sowed on April 13, and transplanted on May 15, with 30 cm row spacing and 13.3 cm plant spacing. Cultivation and management were the same as in the local rice fields.

Cheng's index (Cheng, 1993) and vascular bundle characteristics (Xu et al., 2003b) were used to discriminate indica and japonica. The glume color and leaf pubescence were investigated at heading stage, and five middle stem every line were selected to investigate the number of large and small vascular bundles in panicle neck and second top stem by manual slicing stem, and the ratio of large to small vascular bundles in panicle neck and the ratio of large vascular bundles between the second top stem and panicle neck were counted, with five middle plants per line at ripe stage sampled for indoor analysis after being air dried. The length of the first and second rachis, length/width ratio of spikelets, glume pubescence, and phenol reaction were investigated and the scores were graded based on Cheng's index method.

3 Results

3.1 Changes of Cheng's index under different ecological environments

The population showed continuous variation on frequency distribution either in Sichuan or in Liaoning (Fig. 1). It

suggested that there was no obvious boundary among every segregate phenotype on subspecies characteristics. Because Cheng's index, which ranged from 0 to 24, represented the gradual changes from indica, indica-cline, japonica-cline to japonica, RILSA showed obvious japonicalinous distribution. It was considered that RILSA was more japonicalinous in Sichuan than in Liaoning from the peak value excursion of frequency distribution.

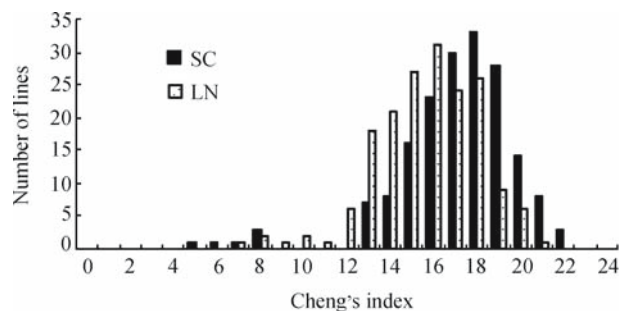


Fig. 1 Frequency distribution of Cheng's index under different environments

Further studies showed that frequency distributions of six subspecies traits were continuously varied both in Sichuan and in Liaoning, but the effects of ecology environments on them were inconsistent. The length of the first and second rachis showed more japonicalinous in Sichuan than in Liaoning, as well as leaf pubescence, but length/width ratio of spikelets and glume color showed indicinalinous in Sichuan than in Liaoning.

The statistical results showed that (Table 1), Cheng's index, length of the first and second rachis and leaf pubescence were greater in Sichuan than those in Liaoning, while length/width ratio of spikelets and glume color were less in Sichuan than those in Liaoning. RILSA was divided into four types such as indica (H), indica-cline (H'), japonica-cline (K') and japonica (K) based on Cheng's index. The analysis showed that indica and indica-cline types showed more indicinalinous while japonica-cline and japonica types showed more japonicalinous in Sichuan than those in Liaoning. The change direction and difference significance were different according to different characteristics. For example, length/width ratio of spikelets in four types as well as the total scores showed more indicinalinous in Sichuan than those in Liaoning, but glume color in indica, indica-cline and japonica-cline types showed more indicinalinous. As to japonica-cline, it showed more japonicalinous in Sichuan than those in Liaoning, and the total score was japonicalinous.

3.2 Changes of relationships of six subspecies characteristics under different ecological environments

As far as the same characteristics are concerned, they showed significant correlations in Sichuan and in Liaoning, which suggested that the effect of ecological environments on the same trait was identical (Table 2), but the extent of correlation

Table 1 Effect of environment on Cheng's index of different subspecies type

Type	Statistical result	GP		PhR		LoR		GC		LP		L/W		ChI	
		SC	LN	SC	LN	SC	LN	SC	LN	SC	LN	SC	LN	SC	LN
H and H'	Mean	1.57	1.98	2.13	2.00	1.07	0.87	1.80	2.87	2.00	1.73	1.93	2.47	10.50	11.92
	Variance	0.45	0.98	2.37	2.43	0.50	1.41	1.60	0.23	3.14	2.96	0.35	0.27	10.56	11.04
	<i>t</i> value	-2.38*		0.94		0.76		-4.07**		1.20		-4.00**		-2.88*	
K'	Mean	1.72	1.81	3.60	3.72	1.95	1.39	2.70	3.02	3.72	2.06	2.35	2.69	16.05	14.68
	Variance	0.62	0.76	0.75	0.55	0.78	0.84	1.21	0.21	0.30	1.04	0.37	0.26	1.21	3.84
	<i>t</i> value	-1.31		-2.68**		4.27**		-3.10**		16.51**		-5.47**		7.34**	
K	Mean	2.49	2.40	3.86	3.82	2.64	1.89	3.49	3.32	3.89	2.45	2.62	2.84	19.00	16.71
	Variance	0.44	0.54	0.15	0.26	1.01	0.90	0.33	0.20	0.13	1.34	0.24	0.17	0.94	2.58
	<i>t</i> value	1.56		1.00		6.28**		2.65**		11.12**		-4.17**		12.06**	
Total	Mean	2.03	2.07	3.58	3.62	2.16	1.55	2.95	3.13	3.64	2.19	2.43	2.73	16.80	15.29
	Variance	0.68	0.76	0.84	0.82	1.06	1.00	1.13	0.23	0.71	1.36	0.35	0.23	7.53	5.86
	<i>t</i> value	-0.97		-1.07		7.11**		-2.58**		17.96**		-7.66**		10.17**	

Note: * and ** stand for significance at $P < 0.05$ and $P < 0.01$ levels, respectively. GP = glume pubescence; PhR = phenol reaction; LoR = length of 1st&2nd rachis; LP = Leaf pubescence; L/W = Length/Width ratio of Spikelets; GC = glume color at heading time. SC = Sichuan; LN = Liaoning. ChI = Cheng's Index. The same for the other tables and figures.

Table 2 Relationships of Cheng's index under different environments

		Sichuan						
		GP	PhR	LoR	GC	LP	L/W	ChI
Liaoning	GP	0.780**	-0.029	0.024	0.122	-0.063	0.158	0.296**
	PhR	0.073	0.892**	0.163*	0.038	0.384**	0.072	0.529**
	LoR	0.123	0.137	0.365**	0.072	0.262**	-0.042	0.319**
	GC	0.333**	-0.044	-0.054	0.559**	0.163*	0.139	0.362**
	LP	0.059	-0.118	0.028	0.348**	0.468**	0.118	0.293**
	L/W	0.124	0.109	0.009	0.160*	0.270**	0.524**	0.335**
	ChI	0.479**	0.336**	0.225**	0.398**	0.541**	0.255**	0.716**

among different traits were different. The correlations of six subspecies traits were not significant except for the glume color and leaf pubescence, the glume color and glume pubescence, the glume color and length/width ratio of spikelets, the length of the first and second rachis, and phenol reaction both in Sichuan and in Liaoning (Table 3). In addition, the leaf pubescence and phenol reaction, the leaf pubescence and length of the first and second rachis, the glume pubescence and length/width ratio of spikelets, and the leaf pubescence and length/width ratio showed significant correlations in Sichuan, which showed no significant correlations in Liaoning. Maybe the subspecies characteristics from typical

indica and typical japonica parent were not randomly segregated and recombined during the genes segregation and recombination in filial generations. The nonrandom phenomenon was greater or less under different ecological environments. As a result, the correlations were significantly different.

3.3 Changes of vascular bundle characteristics under different ecological environments

The effects of ecological environments on the number of large and small vascular bundles in the panicle neck, small vascular bundles in the second top stem, ratio of large to small vascular bundles in the panicle neck, ratio of large vascular bundles between the second top stem and panicle neck were significant except for the number of large vascular bundles in the second top stem (Table 4). To a given experiment plot, the number of large and small vascular bundles in the panicle neck and second top stem were investigated at the same time, with consistent experiment conditions and similar random errors, and there was no significant difference on the number of large vascular bundles in the second stem but the significant difference appeared on other vascular bundle characteristics. Therefore, it could be concluded that the number of large vascular bundles in the second stem was a relatively stable character. This variation was very small under different

Table 3 Correlations of Cheng's index traits

Trait	Location	GP	PhR	LoR	GC	LP	L/W
GP	SC	-					
	LN	-					
PhR	SC	0.040	-				
	LN	-0.027	-				
LoR	SC	0.102	0.176*	-			
	LN	0.021	0.159*	-			
GC	SC	0.180*	0.010	-0.095	-		
	LN	0.243**	0.015	0.096	-		
LP	SC	0.037	0.305**	0.226**	0.284**	-	
	LN	0.016	-0.009	0.066	0.239**	-	
L/W	SC	0.224**	0.051	-0.078	0.213**	0.193*	-
	LN	0.055	0.138	0.023	0.186*	0.137	-

Table 4 Changes of vascular bundle traits under different environments

Trait	Location	Mean	Variance	t value
LVBN	SC	14.59	5.70	-4.27**
	LN	15.14	7.01	
SVBN	SC	24.90	21.14	2.69**
	LN	24.18	22.20	
LVBS	SC	31.89	4.34	1.95
	LN	31.64	4.88	
SVBS	SC	30.19	8.06	6.61**
	LN	29.16	6.12	
RLSVB	SC	0.60	0.01	-6.44**
	LN	0.64	0.01	
RLVB	SC	2.25	0.09	5.41**
	LN	2.15	0.09	

Note: LVBN = Large vascular bundles in panicle neck; SVBN = Small vascular bundles in panicle neck; LVBS = Large vascular bundles in the second top stem; SVBS = Small vascular bundles in the second top stem; RLSVB = Ratio of large to small vascular bundles in panicle neck; RLVB = Ratio of large vascular bundles between the second top stem and panicle neck. The same below.

ecological environments. Analyzed by mean value, the number of large vascular bundles in the panicle neck was less, the number of small vascular bundles in the panicle neck and second top stem was greater, the ratio of large to small vascular bundles in the panicle neck was less and the ratio of large vascular bundles between the second top stem and panicle neck was greater in Sichuan than that in Liaoning.

The ratio of large to small vascular bundles in panicle neck and ratio of large vascular bundles between the second top stem and panicle neck all had continuous distribution (Figs. 2 and 3). It could be concluded that RILSA showed japonicalinous distribution based on the studied results from Xu et al. (2003b). Because the peak value of ratio of large to small vascular bundles in panicle neck distribution tended to be small and the peak value of ratio of large vascular bundles between the second top stem and panicle neck distribution tended to be large, it suggested that RILSA were more japonicalinous in Sichuan than that in Liaoning. It also verified the results of classification based on Cheng's index. As far as the same trait was concerned, similar with Cheng's index, it showed significant correlation both in Sichuan and in Liaoning, which indicated that effect of ecological environments on vascular bundle characteristics was similar (Table 5). There were significant correlations among the vascular bundle characteristics except for the ratio of large to small vascular

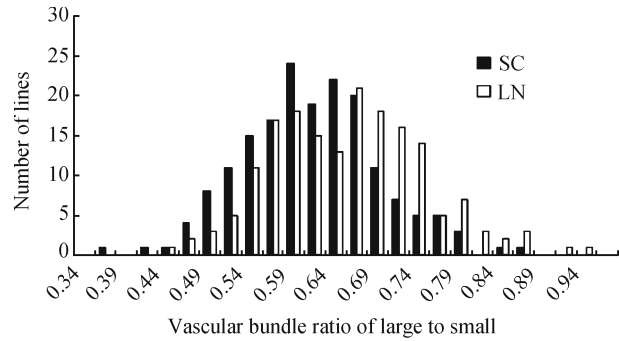


Fig. 2 Frequency distribution of the vascular bundle ratio of large to small under different environments

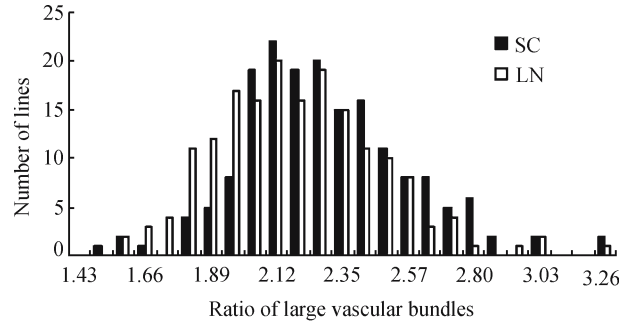


Fig. 3 Frequency distribution of the ratio of large vascular bundles under different environments

bundles in panicle neck and the number of large vascular bundles in panicle neck.

3.4 Changes of relationship of Cheng's index and vascular bundle characteristics under different ecological environments

There were significant correlations among the vascular bundle characteristics and their directions of correlation were the same, whereas the relationships between Cheng's index and vascular bundle characteristics were significantly different (Table 6). The correlation between Cheng's index and vascular bundle characteristics did not reach the significant level in Sichuan, while in Liaoning, Cheng's index and vascular bundle characteristics showed significant correlation except for the ratio of large to small vascular bundles in panicle neck. The reason maybe is that the changes of Cheng's index and

Table 5 Relationships of vascular bundle traits under different environments

Trait		Sichuan					
		LVBN	SVBN	LVBS	SVBS	RLSVB	RLVB
Liaoning	LVBN	0.772**	0.540**	0.618**	0.594**	0.154	-0.652**
	SVBN	0.631**	0.707**	0.663**	0.653**	-0.226**	-0.447**
	LVBS	0.629**	0.550**	0.696**	0.647**	-0.019	-0.430**
	SVBS	0.617**	0.585**	0.717**	0.710**	-0.082	-0.400**
	RLSVB	0.080	-0.304**	-0.160*	-0.172*	0.515**	-0.196*
	RLVB	-0.685**	-0.441**	-0.461**	-0.425**	-0.193*	0.663**

Table 6 Correlations between Cheng’s index and vascular bundle characteristics

	Location	ChI	LVBN	SVBN	LVBS	SVBS	RLSVB	RLVB
ChI	SC	–						
	LN	–						
LVBN	SC	–0.112	–					
	LN	–0.361**	–					
SVBN	SC	–0.057	0.696**	–				
	LN	–0.235**	0.710**	–				
LVBS	SC	–0.125	0.664**	0.700**	–			
	LN	–0.249**	0.746**	0.786**	–			
SVBS	SC	–0.119	0.702**	0.746**	0.757**	–		
	LN	–0.201*	0.723**	0.787**	0.875**	–		
RLSVB	SC	–0.020	0.222**	–0.529**	–0.174*	–0.181*	–	
	LN	–0.048	0.231**	–0.503**	–0.174*	–0.205*	–	
RLVB	SC	0.079	–0.902**	–0.499**	–0.333**	–0.477**	–0.387**	–
	LN	0.348**	–0.907**	–0.524**	–0.467**	–0.485**	–0.386**	–

vascular bundle characteristics were not the same under different environments.

3.5 Comparison of Cheng’s index and changes of vascular bundle characteristics under different ecological environments

The frequency distributions of lines were japonica-cline > japonica > indica-cline > indica in Sichuan, and japonica-cline > indica-cline > japonica > indica in Liaoning (Table 7). The total trend of vascular bundle characteristics changes was indica > japonica for the ratio of large to small vascular bundles in the panicle neck and japonica > indica for the ratio of large vascular bundles between the second top stem and panicle neck. It was consistent with the study results from Xu et al. (2003b), but the boundary of large to small vascular bundles in the panicle neck and the large vascular bundles between the second top stem and panicle neck among indica-cline, japonica-cline and japonica were blurry. The scopes of variation of large to small vascular bundles in the panicle neck and large vascular bundles between the second top stem and panicle neck were japonica-cline > japonica > indica-cline > indica, which was likely correlated with the

frequency distribution, because the more the numbers of lines, the larger the scope of variation. The approximately smooth curves were made to verify this consideration (Figs. 4–7). Obviously, the scopes of variation in both the large to small vascular bundles in the panicle neck and the large vascular bundles between the second top stem and panicle neck were consistent with japonica-cline > japonica > indica-cline > indica, and these curves of frequency distribution were overlapped. So it was inappropriate that the vascular

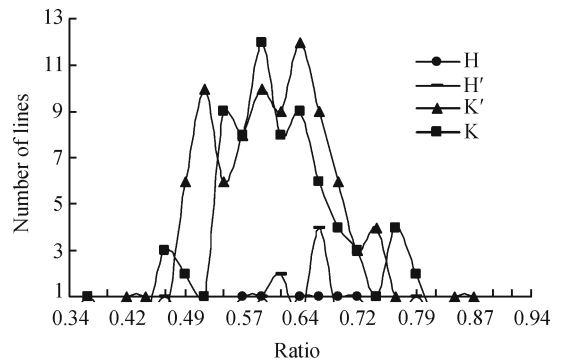


Fig. 4 Frequency distribution of the vascular bundles ratio of large to small in Sichuan

Table 7 Changes of RLVB and RLSVB under different environments

Value	SC				LN			
	H	H'	K'	K	H	H'	K'	K
Mean	2.17	2.39	2.21	2.29	1.99	2.07	2.14	2.37
	0.63	0.62	0.60	0.60	0.69	0.64	0.64	0.64
SD	0.17	0.42	0.30	0.28	0.13	0.26	0.29	0.27
	0.06	0.08	0.08	0.08	0.05	0.08	0.09	0.11
Min	1.93	1.82	1.44	1.57	1.85	1.54	1.63	1.95
	0.56	0.45	0.41	0.34	0.66	0.52	0.43	0.46
Max	2.38	3.25	3.02	3.19	2.15	2.79	2.99	3.26
	0.69	0.77	0.86	0.77	0.77	0.94	0.89	0.82
CV/%	7.95	17.62	13.47	12.29	6.73	12.76	13.53	11.41
	8.85	13.46	13.72	13.87	7.46	12.79	14.69	16.43
Frequency	6	9	88	73	4	40	108	24

Note: For every parameter with two values, the upper is RLVB, lower is RLSVB.

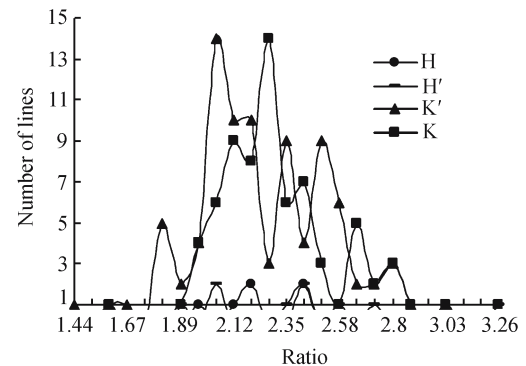


Fig. 5 Frequency distribution of the ratio of large vascular bundles in Sichuan

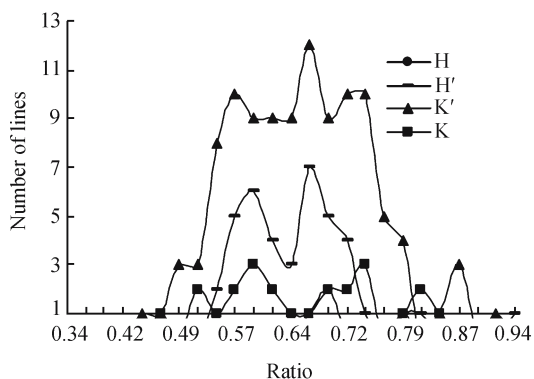


Fig. 6 Frequency distribution of the vacuolar bundles ratio of large to small in Liaoning

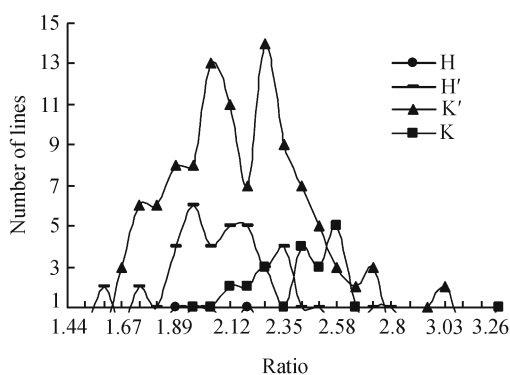


Fig. 7 Frequency distribution of the ratio of large vascular bundles in Liaoning

bundles ratio of large to small and the ratio of large vascular bundles were regarded as the parameters for subspecies classification in rice. The vascular bundle characteristics from typical indica or japonica were recombined in the population derived from the cross between indica and japonica.

4 Discussion

The indica-japonica differentiation stands for the mainstream of genetic differentiation in Asian cultivated rice (Zhou et al., 1988; Wang et al., 1997; Sun et al., 1998; Li et al., 1999), which has obvious differences in morphological characteristics and physiological structures, and these differences come from the natural selection and artificial selection or their long-term interaction. Indica and japonica have different ecotypic adaptabilities adapted to the special geographies and climates respectively. Breeding by crossing indica and japonica is aimed at breaching the boundary between them to create a middle type with elite traits for rice production. However, a large number of cultivars bred by the method of crossing between indica and japonica still retain the typical japonica characteristics in northern China, and additionally, the breeders have not consciously selected the cultivars based on subspecies characteristics. In our experiment, the process of

establishing RILSA could partly simulate the breeding procedure in northern China where the indica-japonica crossing was conducted by multiplying and adding a generation in Hainan Island and breeding in northern China without artificial selection. In fact, only these lines were adapted simultaneously to the geography and climates and the local plant system could survive. For example, among the indica-cline lines, some sensitive to photoperiod could not head normally under the long sunlight condition, and some with poor cold-tolerance could not germinate normally under low temperatures in early spring. Besides, these indica-cline lines could not survive by natural selection. So it was evident that RILSA in our study showed a japonicalinous distribution. We could conclude that the breeding in northern China should also be japonicalinous without artificial selection according to japonica characteristics. When the filial generations of a cross between indica and japonica were sown in Sichuan, why were the indica lines more indicalinous and the japonica lines more japonicalinous? Our argumentation is that the indica grown in low latitude and low altitude belonged to the low-grade type in evolution, while the japonica was the advanced type, originating from the indica that spread to high latitude and high altitude. Actually, it is irreversible for the breeding process from low-grade to high-grade. Through the procedure of multiplication in Hainan Island and breeding in northern China, the indica genes can be reduced, while the japonica genes will be dominant in the filial generations of a cross between indica and japonica. Even if this population was sown in low latitude and low altitude regions with short-sunlight and high temperature conditions it could not be reversible during the process of indica-japonica differentiation. The conclusion should be further verified because of relatively fewer indica and indica-cline lines in RILSA.

Among the filial generations of a cross between indica and japonica, the correlations among subspecies characteristics of Cheng's index, and Cheng's index and vascular bundle characteristics were different under different environments. The reasons maybe were the inherent combination with indica and japonica characteristics (Cheng, 1993) or correlated with the nonrandomness phenomenon between indica loci and japonica loci (Sano and Morishima, 1992; Chen, 1999). In the typical indica or japonica population derived from natural differentiation, subspecies characteristics showed a consistent direction in indica-japonica classification at the same time, and their correlation was significant. Crossing between indica and japonica might break up the originally significant correlation among subspecies characteristics (Mei et al., 1997). The sensitiveness of every trait to environment was different, and the direction and extent of variation were inconsistent. Gene linkage and interaction could lead to phenotypic characteristics to be correlated and embedded, and the effects of environment on the phenotype were different, which would make these characteristics tend to complexity (Li et al., 1999). As a result, the discrimination of indica and japonica should be based on more traits that are not equally important on

classification (Cheng et al., 1984; He et al., 1994; Oka, 1958). The law that the ratio of large to small vascular bundles in panicle neck in indica is greater than that in japonica, and the ratio of large vascular bundles between panicle neck and second top stem in indica is less than that in japonica, is still applied to the typical indica and japonica, but it is inappropriate to be regarded as parameters for subspecies classification in rice because the vascular bundle characteristics are recombined in the population derived from typical indica and japonica crossing. The number and area of vascular bundles showed significant positive correlation with large panicles, which were the anatomical basis of large sink and expedite stream (Huang, 1998). It can be concluded that the northern breeders are supposed to breed the varieties with large panicles, more large vascular bundles in the panicle neck and more primary branches as is done in southern China by the method of crossing between indica and japonica.

Crop ecological adaptability is a long and gradual change process, the effect of different environments on the growth and development is extensive, comprehensive and complex, and furthermore, the experiment results on two plots in one year must have some limitation, which may be different because of different combination of indica-japonica crossing and different crossing method (Xu et al., 1994). For example, Cheng's index and the different ratios of large vascular bundles between the panicle neck and the second top stem of parents in RILSA as well as of large to small vascular bundles in panicle neck were relatively close, which might affect the evaluation of the value of vascular bundles characteristics for subspecies classification. Therefore, more experiments with more indica and japonica crossing populations in wider regions in more years should be done to further verify the conclusions and reveal the effects of ecological environments on indica-japonica differentiation, the relationship between subspecies characteristics and economic characteristics in the future.

5 Conclusions

The RILs (short for RILSA) population derived from a cross between indica and japonica was grown in Liaoning and Sichuan Provinces to compare the subspecies characteristics under different environments. The results showed that the distribution of Cheng's index was japonicalinous. The population was more japonicalinous in Sichuan than in Liaoning as a whole. Further analysis indicated that the indica and indica-cline as well as the japonica and japonica-cline in Sichuan were more indicalinous and more japonicalinous than those in Liaoning, respectively. The different environments may have different effects both on six traits of Cheng's index and on the correlations between the six traits, the Cheng's index and the vascular bundle traits because of different characteristics. The vascular bundle characteristics of RILs in different environments changed remarkably. The

number of large vascular bundles in the panicle neck was smaller in Sichuan than in Liaoning, but the number of small vascular bundles in the panicle neck and the second top stem was larger in Sichuan than in Liaoning. The ratio of large to small vascular bundles in the panicle neck decreased while the ratio of large vascular bundles between the second top stem and panicle neck increased in Sichuan. In general, though there was a general trend that the vascular bundles ratio of large to small in the indica was greater than that in the japonica, and the ratio of large vascular bundles in the indica was less than that in japonica, it was inappropriate to be viewed as the parameters for subspecies classification in rice. The vascular bundles characteristics from typical indica or japonica were recombined in the population derived from indica and japonica crossing.

Acknowledgements This research was supported by the National Natural Science Foundation of China (Grant No. 30370866) and the Doctor Foundation of Ministry of Education (No. 20050157001).

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