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Correlation and Regressive Model Between Spikelet Fertilized Rate and Temperature in Inter-Subspecific Hybrid Rice

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Abstract: To study the sensitivity of inter-subspecific hybrid rice to climatic conditions, the spikelet fertilized rate (SFR) of four types of rice including indica-japonica hybrid, intermediate hybrid, indica and japonica were analyzed during 2000-2004. The inter-subspecific hybrids showed lower SFR, and much higher fluctuation under various climatic conditions than indica and japonica rice, showing the inter-subspecific hybrids were sensitive to ecological conditions. Among 12 climatic factors, the key factor affecting rice SFR was temperature, with the most significant factor being the average temperature of the seven days around panicle flowering (T_7). A regressive equation of SFR-temperature by T_7, and a comprehensive synthetic model by four important temperature indices were put forward. The optimum temperature for inter-subspecific hybrids was estimated to be 26.1-26.6℃, and lower limit of safe temperature to be 22.5-23.3℃ for panicle flowering, showing higher by averagely 0.5℃ and 1.7℃, respectively, to be compared with indica and japonica rice. This suggested that inter-subspecific hybrids require proper climatic conditions. During panicle flowering, the suitable daily average temperature was 23.3-29.0℃, with the fittest one at 26.1-26.6℃. For an application example, optimum heading season for inter-subspecific hybrids in key rice growing areas in China was as same as common pure lines, while inferior limit for safe date of heading was about a ten-day period earlier than those of common pure lines.
Key words: fertilization; inter-subspecific hybrid rice; regression; temperature; climatic conditions

Hybrid rice commonly shows heterosis in their biomass or grain yield. Generally, inter-subspecific hybrids display a tendency of stronger heterosis than intra-subspecific ones. However, inter-subspecific hybrids were more sensitive to ecological conditions due to the genetic differences of the two parents, especially for their seed setting [1-3], which has been a main obstacle to utilize such hybrids. The spikelet fertility (or spikelet fertilized rate, SFR) of common indica-japonica hybrids was as low as 0-40%. The sterility has been mainly attributed to an allelic interaction at locus *S5* on chromosome 6, and the problem has been mitigated by a neutral allele *S5-n* (so called wide compatibility gene, WCG) at the locus in some varieties [4-5]. For a given hybrid, its SFR is determined by the plant physiological characters such as source of photosynthetic products or yield sink and so on [6], and another important factors, i.e. ecological conditions, especially climatic conditions such as temperature and solar radiations.

In the studies on effects of climatic factors on rice SFR, there were numerous reports for pure lines or intra-subspecific hybrids [7-12]. Contrastively, few reports were for inter-subspecific hybrids. Furthermore, those studies were only using simple factor such as daily average temperature [2, 13]. To further understand the relationship between climatic factors and rice SFR, 22 rice hybrids or varieties, which were classified into four types as indica-japonica hybrid, intermediate hybrid, indica or japonica, were used to investigate the SFRs of panicles headed under various climatic conditions during July to October in 2000-2004, by analyzing the relationships between 12 corresponding climatic factors including temperature, sunshine hours, rainfall and relative humidity and SFR. Moreover, a regressive equation and a synthetic model of SFR-temperature for four types of rice were put forward. On the basis of temperature effect on SFR in inter-subspecific hybrid rice, a strategy for determining suitable planting area or growing conditions for such hybrids was suggested, and an application example of the optimum and safe heading seasons for growing inter-subspecific hybrid rice in three different

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ecological areas of South China according to the multiple-year climate data was given.

MATERIALS AND METHODS

Plant materials and treatments

Twenty-two rice hybrids or varieties were used, which were classified into four types as indica-japonica hybrid, intermediate hybrid (javanica /indica or japonica/javanica hybrid), indica or japonica. Both the types of indica-japonica hybrids and intermediate hybrids were considered as inter- subspecific hybrids.

The indica-japonica hybrids (indica/japonica or japonica/indica) were A12 (Kasalath/Akihikari), A23 (Kanou 262/Kasalath), A38 (Milyang 21/Akihikari), A122 (Ketan Nangka/IR36), A172 (3037/02428), A173 (Nanjing 14/02428) and A175 (Nanjing 11/ 02428).

The intermediate hybrids (javanica/indica or japonica/javanica) were Liangyoupeijiu (Pei'ai 64S/ 9311), Liangyou E32 (Pei'ai 64S/E32), Liangyou 106 (Pei'ai 64S/Z3106), Liangyou 108 (Pei'ai 64S/Z3108) and Siyou 422 (731A/Lunhui 422).

The indica type (or indica/indica hybrid) were 9311, IR36, Kasalath, Nanjing 11 and Shanyou 63 (Zhenshan 97A/Minghui 63).

The japonica type were 02428, 95122, Ketan Nangka (KN), Kanou 262 and Akihikari.

The experiments were carried out in 2000, 2001, 2003 and 2004 at the rice experimental field of Jiangsu Academy of Agricultural Sciences (JAAS) (32°02′ N, 118°51′ E), Nanjing, China. All the plant materials were grown under the same conditions such as fertilizer and water managements besides the sowing date. For each year, from 20th April to 9th July or 30th April to 19th July, each rice hybrid or variety was sown on five dates at a 20-day interval. During the heading period (July to October), all flowering panicles were labeled, and their SFR were determined on five panicles after maturity. Every year, for each hybrid or variety, the panicles were sampled 10-15 times at the 3-5 days interval for determining the SFR. The SFR was determined by the rate of spikelets with starch accumulation.

Data and analysis

The corresponding climate data in the experimental site, including daily average minimum and maximum temperatures, sunshine hours, rainfall and relative humidity, were provided by the weather station of JAAS, and the climate data of 42 years (1951-1992) in 28 cities of key rice growing areas in China were provided by China Meteorological Administration.

The correlation analyses between SFR and 12 climatic factors were surveyed. The climatic conditions during flowering of a panicle were average temperatures T_5 (the average temperature of the five days around a panicle flowering, the 'five days' means the duration from two days before to two days after the flowering day) and T_7 (the average temperature of the seven days around a panicle flowering, the 'seven days' means the duration from three days before to three days after the flowering day); average daily maximum temperatures of the five and seven days, T_{M5} and T_{M7}; average daily minimum temperatures of the five and seven days, T_{m5} and T_{m7}; average sunshine hours of the five and seven days, S_5 and S_7; average daily rainfall of the five and seven days, R_5 and R_7; average relative humidity of the five and seven days, RH_5 and RH_7. By correlation and regression analyses, models between SFR and climatic factors for each type of rice could be established [14].

For an application example, according to the present experiment and the climate data from 1951-1992 of key rice growing areas in China, a strategy for determining the optimum and safe heading date of inter-subspecific hybrid rice planting in such areas was put forward.

RESULTS

Differences of SFR in four types of rice

Under the experimental conditions, the SFRs were largely different in four types of rice (Table 1). The indica-japonica hybrids showed the lowest SFR at 62.36±16.68%, and intermediate hybrids at 73.57± 9.85%, while indica and japonica rice displayed higher SFRs at 78.87±14.15% and 80.63±3.72%, respectively. The SFRs of indica- japonica hybrids and intermediate hybrids were lowered by 17.39 and 6.18 percent points, respectively, comparing with the averaged values of indica and japonica rice (including their parents) (Table 1). This confirmed that inter-subspecific rice hybrids have lower SFRs than indica

Table 1. Spikelet fertilized rate (SFR) under various natural conditions of four types rice.

Rice type	Sampling times	Average SFR (%)	The highest SFR (%)	*CV* of SFR (%)	Rice type	Sampling times	Average SFR (%)	The highest SFR (%)	*CV* of SFR (%)
indica					japonica				
9311	35	74.80	92.66	17.15	02428	21	79.91	97.61	23.19
IR36	57	74.60	96.94	23.63	95122	30	83.30	100.00	22.04
Kasalath	17	91.09	98.20	5.68	KN	32	81.82	97.00	21.41
Nanjing 11	37	74.03	90.76	17.52	Kanou 262	20	79.52	95.30	26.28
Shanyou 63	44	79.81	93.52	13.52	Akihikari	34	78.60	98.76	26.50
Mean		78.87±14.15 AB	94.42±6.04 b	15.50	Mean		80.63±3.72 A	97.73±3.49 a	23.88
indica-japonica hybrid					Intermediate hybrid				
A12	15	76.36	97.20	37.39	Liangyoupeijiu	54	65.36	96.00	36.02
A23	21	65.96	97.39	50.03	Liangyou E32	51	77.28	96.60	24.37
A38	38	53.37	85.09	41.42	Liangyou 106	16	72.47	88.59	23.68
A122	41	62.77	92.46	38.15	Liangyou 108	13	77.57	86.55	8.31
A172	52	52.30	93.40	60.80	Siyou 422	42	75.19	96.80	26.93
A173	42	67.53	92.26	38.10	Mean		73.75±9.85 B	92.91±9.67 b	23.86
A175	51	58.25	96.67	56.69					
Mean		62.36±16.68 C	93.50±8.48 b	46.08					

Means followed by different lowercase and uppercase letters denote significant differences on average SFR and the highest SFR between the types of rice at P=0.05 and P=0.01, respectively.

and japonica rice, and indica-japonica hybrids, which have larger genetic differences between the parents, performed lower SFRs than intermediate ones. Interestingly, of the four types of rice, the highest SFRs were similar, implying that if only the growing conditions were suitable, indica-japonica and intermediate hybrids could exhibit high SFRs as indica or japonica rice. On the other hand, the coefficient of variation (*CV*) of SFR in indica-japonica hybrids was 46.08%, much higher than those of the other three types, showing the lower SFR stability in indica-japonica hybrids (Table 1).

Survey of climatic factors affecting SFR

Among the 12 factors, six temperature factors (T_5, T_7, T_{m5}, T_{m7}, T_{M5} and T_{M7}) showed significant correlations with SFR (P<0.01), which suggested that temperature was the key factor affecting rice SFR (Table 2). Of the six temperature factors, a tendency of coefficients of correlation between the temperatures and SFR was shown as $T_7 > T_5$, $T_{M7} > T_{M5}$ and $T_{m7} > T_{m5}$, indicating that the average temperatures of the seven days around a panicle flowering were more important than those of the five days. Other six factors (S_5, S_7, R_5, R_7, RH_5 and RH_7) showed sporadically significant correlations with SFR in some hybrids or varieties. The correlation coefficients of the four rice types showed an order of indica-japonica hybrid ＞ japonica ＞ intermediate hybrid ≈ indica (Table 2).

Effect of temperature on SFR

According to the above results, the factor of T_7 was chosen to analyze the effect of temperature on rice SFR. The seven temperature ranges, i.e. ≤20 ℃, 20.1-22.0℃, 22.1-24.0℃, 24.1-26.0℃, 26.1-28.0℃, 28.1-30.0℃ and >30.0℃ were classified, of which ≤24℃, 24.1-28.0℃ and >28.0℃ were as low, middle and high temperature ranges, then all data were averaged within such ranges. The result showed that the differences of SFR between four types of rice were larger at low temperature range, while smaller at middle temperature range (Table 3). Within a common low temperature range, SFR showed a tendency of indica-japonica hybrid < intermediate hybrid < indica <japonica, suggesting the lowest tolerance to low temperature of indica-japonica hybrid.

Using the factor T_7 to regress equation of SFR (Y)-temperature (T), the following equations which all showed significant regression effects were obtained (P<0.01, Fig. 1).

$Y_{\text{indica-japonica hybrid}} = -0.9913T_7^2 + 51.834T_7 - 599.70$ ($R^2 = 0.617^{**}$, n=151);

$Y_{\text{intermediate hybrid}} = -0.6833T_7^2 + 36.334T_7 - 401.41$ ($R^2 = 0.456^{**}$, n=176);

Table 2. Coefficients of correlation between spikelet fertilized rate and climatic factors.

Rice material	T_5	T_7	T_{M5}	T_{M7}	T_{m5}	T_{m7}	S_5	S_7	R_5	R_7	RH_5	RH_7
indica-japonica hybrid												
A12	0.912**	0.941**	0.870**	0.915**	0.925**	0.965**	0.503*	0.616*	0.222	0.250	0.386	0.361
A23	0.881**	0.883**	0.787**	0.798**	0.877**	0.898**	0.211	0.284	0.257	0.247	0.063	0.145
A38	0.760**	0.762**	0.645**	0.642**	0.518**	0.526**	0.437**	0.487**	0.619**	0.635**	0.526**	0.517**
A122	0.601**	0.644**	0.719**	0.727**	0.520**	0.586**	0.509**	0.510**	0.240	0.278	0.274	0.395**
A172	0.783**	0.794**	0.680**	0.689**	0.817**	0.834**	0.279*	0.273*	0.200	0.255	0.066	0.112
A173	0.748**	0.796**	0.664**	0.759**	0.640**	0.684**	0.310*	0.496**	0.070	0.089	0.170	0.209
A175	0.654**	0.720**	0.652**	0.724**	0.598**	0.676**	0.160	0.286*	0.197	0.172	0.115	0.161
Mean	0.763	0.791	0.717	0.751	0.699	0.738	0.344	0.422	0.258	0.275	0.229	0.271
Intermediate hybrid												
Liangyoupeijiu	0.744**	0.776**	0.743**	0.779**	0.690**	0.728**	0.170	0.252	0.126	0.109	0.189	0.211
Liangyou E32	0.568**	0.597**	0.495**	0.544**	0.574**	0.605**	0.232	0.267	0.138	0.092	0.178	0.240
Liangyou 106	0.609**	0.649**	0.431	0.522*	0.491*	0.494*	0.333	0.511*	0.723**	0.487*	0.502*	0.641**
Liangyou 108	0.489	0.505	0.474	0.532*	0.414	0.344	0.396	0.497	0.650*	0.722**	0.632*	0.310
Siyou 422	0.638**	0.637**	0.539**	0.546**	0.657**	0.681**	0.044	0.169	0.242	0.179	0.197	0.216
Mean	0.610	0.633	0.536	0.585	0.565	0.570	0.235	0.339	0.376	0.318	0.340	0.324
indica												
9311	0.387*	0.420*	0.440**	0.491**	0.440**	0.446**	0.096	0.313	0.310	0.344*	0.270	0.218
IR36	0.757**	0.788**	0.689**	0.724**	0.679**	0.728**	0.435**	0.511**	0.149	0.136	0.079	0.057
Kasalath	0.748**	0.763**	0.755**	0.808**	0.632**	0.622**	0.241	0.066	0.332	0.342	0.118	0.236
Nanjing 11	0.700**	0.729**	0.691**	0.738**	0.447**	0.526**	0.415**	0.484**	0.406*	0.386*	0.296	0.348*
Shanyou 63	0.387**	0.279	0.268	0.335*	0.359*	0.433**	0.111	0.110	0.239	0.244	0.312*	0.320*
Mean	0.596	0.596	0.569	0.619	0.511	0.551	0.260	0.297	0.287	0.290	0.215	0.236
japonica												
02428	0.605**	0.600**	0.444*	0.480*	0.534*	0.506*	0.361	0.345	0.257	0.480*	0.394	0.413
95122	0.542**	0.628**	0.518**	0.641**	0.432*	0.490**	0.225	0.323	0.129	0.169	0.398*	0.393*
KN	0.798**	0.835**	0.822**	0.873**	0.604**	0.686**	0.437*	0.630**	0.212	0.318	0.383*	0.342
Kanou 262	0.855**	0.861**	0.839**	0.862**	0.793**	0.845**	0.285	0.479*	0.202	0.266	0.110	0.142
Akihikari	0.747**	0.723**	0.701**	0.665**	0.541**	0.563**	0.562**	0.586**	0.432**	0.411*	0.204	0.184
Mean	0.709	0.729	0.665	0.704	0.581	0.618	0.374	0.473	0.246	0.329	0.298	0.295

**$P<0.01$, *$P<0.05$.

Y_{indica}= $-0.7004T_7^2+36.332T_7-386.92$ ($R^2 = 0.374^{**}$, n=190);

$Y_{japonica}$= $-0.9466T_7^2+48.755T_7-536.71$ ($R^2=0.521^{**}$, n=137).

Some theoretical and actual SFRs were shown in Table 4, the actual SFRs and temperatures were similar to the theoretical values, which confirmed the availabilities of such equations. For an example, at 23.5℃ and 29℃, the theoretical SFRs of indica rice were 80.09% and 77.67%, while the actual SFRs were 82.41±18.40% and 78.00±20.03%, respectively (Table 4).

From the above four equations, temperatures at maximum and 70% of SFRs were calculated as the optimum and safe temperatures, respectively (Table 5). The results showed that, to be compared with the values of indica and japonica rice, indica-japonica and intermediate hybrids had slightly higher optimum temperatures by increasing 0.5℃ averagely, similar to upper limits of safe temperatures, while lower limits of safe temperature by increasing 1.7℃ on average, indicating the sensitivity to lower temperature of inter-subspecific hybrids.

The fluctuating rates of SFR (Y') for variation at a temperature (T) could be calculated from the following four equations.

$Y'_{indica\text{-}japonica\ hybrid} = -1.9826T_7+51.834$;

$Y'_{intermediate\ hybrid} = -1.3666T_7+36.334$;

$Y'_{indica} = -1.4008T_7+36.332$;

$Y'_{japonica} = -1.8932T_7+48.755$.

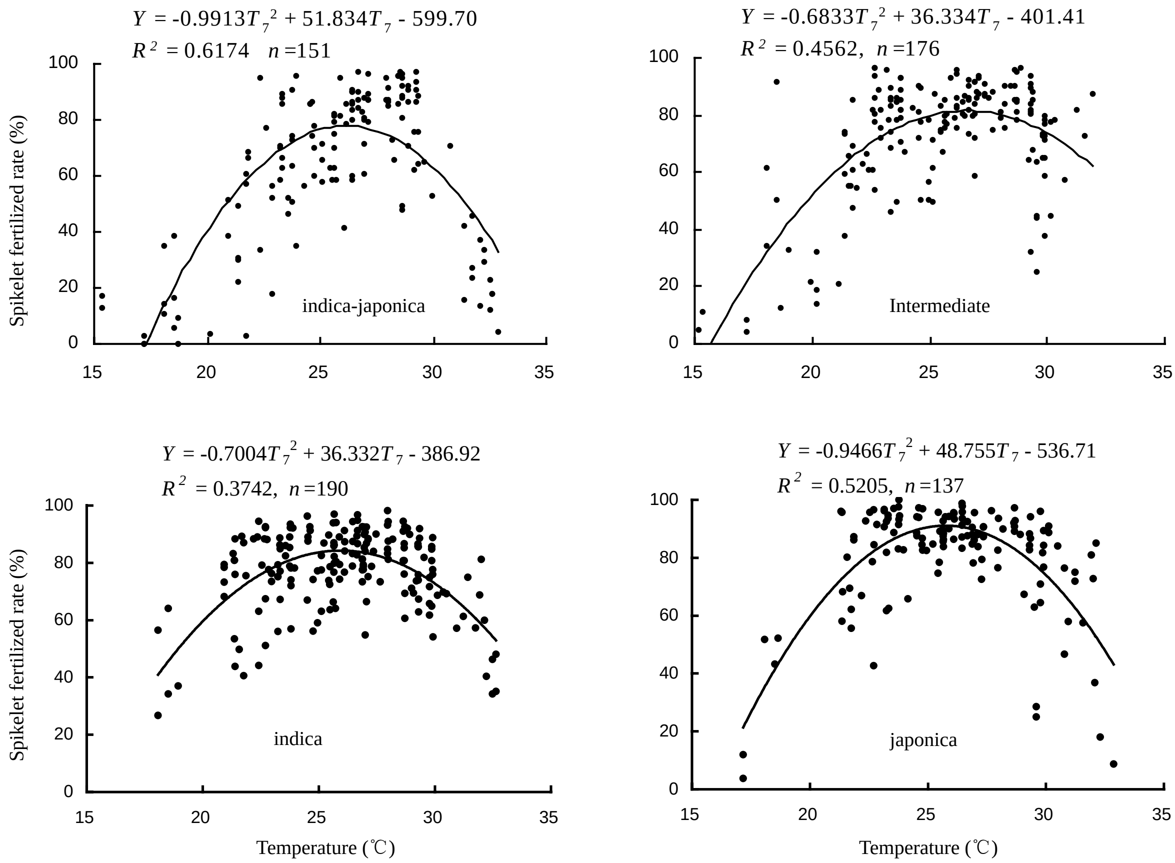

Fig. 1. Correlations between spikelet fertilized rate and temperature for four types of rice.

Table 3. Spikelet fertilized rate of four type rice in different temperature ranges.

Rice type and item	≤20℃	20.1-22.0℃	22.1-24.0℃	24.1-26.0℃	26.1-28.0℃	28.1-30.0℃	>30℃
indica-japonica hybrid							
Sample number	22	18	51	32	51	52	34
Average SFR (%)	12.05	41.14	56.96	68.56	80.41	76.62	43.21
SD	13.96	27.48	27.87	20.16	12.80	18.65	24.94
Intermediate hybrid							
Sample number	11	16	39	25	37	41	7
Average SFR (%)	30.35	51.54	78.66	74.70	84.24	75.26	71.46
SD	27.67	21.26	12.78	12.39	7.37	17.45	14.99
indica							
Sample number	5	16	37	34	45	38	15
Average SFR (%)	43.75	71.76	78.78	79.87	84.68	78.66	58.16
SD	15.83	15.98	12.44	11.13	8.33	10.48	14.57
japonica							
Sample number	5	10	26	26	32	23	15
Average SFR (%)	32.61	75.89	86.47	87.53	88.66	79.28	63.45
SD	23.01	15.15	13.83	7.13	6.39	19.22	25.57

Table 4. Comparison between theoretical and actual spikelet fertilized rate of four types rice.

Rice type	23.5℃			29.0℃		
	Theoretical SFR (%)	Actual SFR (%)	Actual temperature (℃)	Theoretical SFR (%)	Actual SFR (%)	Actual temperature (℃)
indica-japonica hybrid	70.95	67.25±48.53	23.6±0.6	69.80	71.48±46.43	29.1±0.5
Intermediate hybrid	75.09	81.69±13.90	23.7±0.5	77.62	72.73±32.52	29.1±0.6
indica	80.09	82.41±18.40	23.6±0.6	77.67	78.00±20.03	29.0±0.6
japonica	86.27	92.91±6.93	23.7±0.4	81.09	78.97±12.23	29.0±0.7

Table 5. Temperature index for four types of rice.

Index	indica-japonica hybrid	Intermediate hybrid	indica	japonica
Optimum temperature (℃)	26.1	26.6	25.9	25.8
Safe temperature (℃)				
Lower limit	23.3	22.5	21.4	21.0
Upper limit	29.0	30.7	30.5	30.5

Table 6. Fluctuating rate of SFR for per unit temperature increase at a temperature (%).

Temperature (℃)	indica-japonica hybrid	Intermediate hybrid	indica	japonica
20	12.18	9.00	8.32	10.89
22	8.22	6.27	5.51	7.10
24	4.25	3.54	2.71	3.32
26	0.29	0.80	-0.09	-0.47
28	-3.68	-1.93	-2.89	-4.25
30	-7.64	-4.66	-5.69	-8.04
32	-11.61	-7.40	-8.49	-11.83

The fluctuating rate of SFR (fluctuation of SFR for per unit variation of temperature) for variation at a temperature was shown in Table 6. It was found that all the four types of rice had small SFR fluctuations within 24-28℃, while indica-japonica hybrids and japonica rice exhibited higher fluctuations when temperatures were outside the range.

Synthetic model of SFR-temperature for four types of rice

To describe precisely the multiple relationships between climatic factors and SFR, a model was used further to regress the effects of all the 12 factors on SFR. By a stepwise analysis and based on the highest correlation coefficient, the synthetic models finally consisted of four important temperature indices, T_7, T_{M7}, T_{m7} and ΔT_5 were obtained, which were shown in Table 7. The models had high R^2 (P<0.0001) for describing the complex effects of temperature factors on rice SFR. If only the key temperature indices during rice heading period were given, the rice SFR would be mainly calculated by the synthetic model.

DISCUSSION

Effects of climatic factors on rice SFR

At a given genetic background, spikelet fertilized rate (SFR) of rice was mainly affected by the plant physiological characters and climatic factors such as temperature during flowering period. The physiological characters affecting the SFR were starch accumulation, ATP content and stigma activity and so on, which were also affected weightily by the climatic factors. The yield sink and photosynthetic products mainly influenced the growth of fertilized spikelets [6].

The analyses of the correlations between SFR and 12 climatic factors showed that temperature was the key factor affecting rice fertilization, and the most

Table 7. Synthetic model of SFR-temperature for four types rice.

Rice type	Model	Effect of model			
		F	P	R^2	Number
indica-japonica hybrid	$Y = -0.7887\times T_7^2+28.1057\times T_7+3.3849\times T_{M7}+11.4318\times T_{m7}+0.6568\times \Delta T_5-490.1674$	46.46	0.0001	0.4777	260
Intermediate hybrid	$Y = -0.6594\times T_7^2+22.9626\times T_7+0.5276\times T_{M7}+11.5494\times T_{m7}+3.3346\times \Delta T_5-373.3867$	33.67	0.0001	0.4976	176
indica	$Y = -0.6886\times T_7^2+28.9650\times T_7+2.5518\times T_{M7}+4.0713\times T_{m7}+1.1262\times \Delta T_5-380.5425$	25.54	0.0001	0.4097	190
japonica	$Y = -0.9032\times T_7^2+35.4615\times T_7+5.4233\times T_{M7}+5.6101\times T_{m7}-0.0753\times \Delta T_5-510.7004$	32.70	0.0001	0.5552	137

T_7, T_{M7}, T_{m7} denoted means of average, maximum and minimum temperatures of the seven days around flowering, respectively. $\triangle T_5$ denotes average value of temperature fluctuation of five days around flowering.

important index was T_7, the average temperature of the seven days around flowering. The averaged maximum and minimum temperatures of the seven days around flowering (T_{M7} and T_{m7}) showed the same tendency to T_7 for their similar performances as the average temperature. In our studies, the synthetic models of SFR-temperature for the four types of rice were established, which consisted of temperature indices of average, maximum, minimum and fluctuation to describe the complex effects of temperature on SFR.

Sensitivity of SFR to temperature in different rice types

The present experiment confirmed that under suitable climatic conditions, the highest SFRs of four types rice were similar, while average values and their variances differed under various climatic conditions. The inter-subspecific hybrids showed a lower average SFR and a higher variance than indica or japonica rice. It implied that SFR and its stability were lower in inter-subspecific hybrid than in indica or japonica rice. Among the two types of inter-subspecific hybrids, the indica-japonica hybrid, the larger one in genetic difference of the parents, showed lower SFR and its stability than the smaller one, intermediate hybrid, suggesting the importance of genetic difference of parents on SFR in hybrid rice [6, 15].

The coefficients of correlation between SFR and the temperature showed a tendency of indica-japonica hybrid＞japonica＞intermediate hybrid ≈ indica, and the effects of lower temperature on SFR showed an order of indica-japonica hybrid＞intermediate hybrid＞indica＞japonica. The present results implied that inter-subspecific hybrids were more sensitive to lower temperature than indica or japonica rice, and among them, intermediate hybrids were relatively easy to be exploited in commercialized for their strong heterosis and relatively higher SFR and its stability [16]. Actually, among the five intermediate hybrids, four hybrids have been registered by the government and allowed to be commercialized. Two intermediate hybrids, Liangyoupeijiu (Pei'ai 64S/9311) and Liangyou E32 (Pei'ai 64S/E32), which were bred by the author's research group and well known as the pioneer of 'Super Hybrid Rice', showed relatively stable SFR and high yielding potential. During 1999-2000, in China National Test for Super Hybrid Rice, Liangyoupeijiu exhibited average grain yield over 10.5 t/ha in 38 sites. The breeding and utilization of Liangyoupeijiu was selected as the first headline of 'The top ten news of science and technology achievement' in China in 2000, and awarded the China National Prize on Technique Innovation and Dupont Innovation Prize. The hybrid has been registered in six provinces and nation of China as the first two-line hybrid, and had been popularized over six million hectare during 1999-2006 in wide ecological areas from north latitude of 0° to 35° in southern regions of China and southeast Asia, e.g., Vietnam, Philippines and Indonesia. Liangyou E32 had been planted from north latitude of 12^o to 35^o and registered in Vietnam in 2001. In 1999, it yielded 17.1 t/ha in a 720 m^2 plot in Yunnan Province, China, which made a yield record of hybrid rice of the time, and reached a grain yield level of 100 kg/ha per day proposed for super high-yielding hybrid rice [17]. In terms of both hybridization and morphological improvement, the hybrid offered a model of plant ideotype for super hybrid rice in the *Science* [18]. On the other hand, typical indica-japonica hybrids showed difficult to commercial production for their higher safe temperature and fluctuation at lower temperature. A hybrid, Yayou 2 (A172, 3037/02428), which also bred by the author's research group in the early 1990s, showed a high yield of 10.5 t/ha and was even planted over 5000 ha. However, it failed to be widely commercialized for its severely fluctuated seed setting by temperature and other ecological conditions around flowering. The hybrid showed seed setting rate of about 90% and displayed a very strong heterosis in grain yield under a favorable condition. However, its seed setting rate was significantly reduced to 20% owing to a low daily average temperature below 24℃ [13]. Thus, it was difficult to plant such hybrids for commercialization in areas north than 32°N, where the temperature is unstable during the season of the rice heading.

To exploit the strong heterosis of inter-subspecific hybrids, especially for indica-japonica hybrids, it is necessary to overcome the lower and unstable SFR caused by its sensitivity to ecological conditions. In the previous studies, the authors found that SFR and its stability decreased by lower temperature in inter-subspecific hybrids was mainly caused by the decrease of pollen fertility, and noted that at a relative lower temperature, the female gametes possessed viability while the pollen displayed lower viability [1-2]. Up to date, several male gamete abortion genes or genes related to lower temperature sensitivity have been mapped [19-21]. Lower temperature caused abortion or decreased the activity of pollen possessing the sensitivity alleles [1, 3, 6, 22]. Increasing the tolerance to lower temperature would be an important approach to increase SFR and its stability in inter-subspecific hybrid breeding. There have been found the alleles to mitigate the sensitivity to climatic conditions for heterozygotes in several rice varieties [23]. Introducing such alleles to parents would be effective to mitigate the sensitivity to climatic conditions in hybrid, and increase SRF and its stability of inter-subspecific hybrids [1, 24].

In the previous studies, the authors reported the effects of lower temperature on the reduction of SFR and its stability in inter-subspecific hybrids [2, 16]. The present study proved that high temperature also decreased rice fertilization [8-9], and the equations provided the optimum and safe temperatures for four types of rice. It would be useful to guide planting such hybrids in suitable growing areas or developing ecological techniques to increase SFR for such hybrids.

Temperature indices and safe date for heading of inter-subspecific hybrid rice

The optimum temperatures and lower limit of safe temperatures for flowering of inter-subspecific hybrids were estimated to be 26.1-26.6℃ and 22.5-23.3℃, respectively, or higher by 0.5 and 1.7℃ than averaged values of indica and japonica rice [2], and those of typical indica-japonica hybrids were higher than those of intermediate hybrids.

The results showed that growing inter-subspecific hybrids required proper climatic conditions. To utilize the strong heterosis of inter-subspecific hybrids, it is necessary to understand the relationship between the SFR and climatic conditions, find out the suitable growing conditions, and plan a suitable ecological area and growing seasons for such hybrids [25].

For an application example, in south China rice growing areas, the extremity temperature about 30℃ (daily averaged value) appeared usually in the middle July to the early August, whereas the temperature was higher from north to south in autumn. The inferior limit for safe date of rice heading would be delayed from north to south. Based on the data in 1951-1992, with a daily average value of 26-27℃ as the optimum temperature, the fittest season (the frequency of typhoon was considered as well) for heading of inter-subspecific hybrids was concluded as the following: The second and third ten-day periods in September for south China (<27°N), the last ten-day period in August to the first ten-day period in September for the Yangtze River Valley (27-33°N), and the middle August for areas between the Yellow River and Huai River (>33°N). The fittest seasons were almost the same as that of indica or japonica pure lines. However, with a daily average value of 23.5℃ as the lower limit of safe temperature, the inferior limit for safe date of heading in inter-subspecific hybrids was about a ten-day period earlier than common pure lines, i. e., the last ten-day period in September to the first ten-day period in October in south China (<27°N), and the first or second ten-day periods in September in the Yangtze River Valley (27-33°N), and more early as in the last ten-day period in August for areas between the Yellow River and Huai River (>33°N) [25].

It is noticeable that the experiments were carried out in an ecologically specific place, Nanjing (32°02' N, 118°51' E), which might limit the efficiency of the model between SFR and temperature. Moreover, besides the temperature, rice SFR would be affected by other ecological factors such as sunshine as well, and the optimum temperature for rice SFR might show a little difference in other dissimilar ecological areas. Thus, additional experiments might be needed in such areas for determining the fittest cropping season and safe date for heading of rice, and then choosing suitable seeding and transplanting dates.

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