An Analysis on the Heat Resistance of Rice Germplasm Resources during Flowering Period

Zhongping ZHA[1,2], Desuo YIN[1,2], Bingliang WAN[1,2,3], Chunhai JIAO[4*]

1. Food Crop Research Institute, Hubei Academy of Agricultural Sciences, Wuhan 430064, China; 2. Hubei Key Laboratory of Crop Germplasm and Genetic Improvement, Wuhan 430064, China; 3. Hubei Collaborative Innovation Center for Grain Industry, Jingzhou 434025, China; 4. Hubei Academy of Agricultural Sciences, Wahan 430072, China

Abstract 56 Chinese rice core germplasm resources, 18 foreign rice germplasm resources and 6 restorer lines are subjected to high temperature stress during flowering period. Based on relative spikelet fertility rate, rice heat resistance is evaluated. The results show that different resistance to high temperature exists in different varieties, and 6 new rice varieties present high heat resistance.

Key words Rice (*Oryza sativa* L.), Germplasm resources, Flowering period, Heat resistance

1 Introduction

Rice (*Oryza sativa* L.) is a major food crop in China, and its yield is related to national food security. The booting and flowering period of rice is from July to August, when rice is vulnerable to high temperature stress. During this period, rice is the most sensitive to temperature and the appropriate temperature for rice development and growth is 25 to 30℃. When the average daily temperature≥ 32℃ and the maximum daily temperature≥ 35℃, it will cause heat damage on rice, resulting in decreased pollen activity, increased blighted seed and decreased setting rate[1 – 3]. A large number of studies showed that anther not cracking, the decease in the number of pollen on stigma, the decline in the pollen germination rate are the main reasons for the infertility of spikelets caused by high temperature. Rang *et al.*[4] found that after the high-temperature-sensitive variety Moroberekan suffered from high temperature stress during the flowering period, there would be a significant decline in the anther dehiscence rate, number of pollen scattered on the stigma and number of pollen germinating on the stigma, resulting in significantly reduced spikelet fertility and setting percentage. Carrying out the heat resistance identification of rice germplasm resources during the flowering period, screening heat-resistant germplasm, and developing heat-resistant rice varieties, is an effective measure to prevent rice yield reduction caused by high temperature. Some high heat-resistant varieties have been identified at home and abroad, such as N22, T226, 996 and R1056[5 – 8]. Currently, the study progress on the use of heat-resistant germplasm for rice's heat resistance improvement is very slow, because it lacks suitable heat-resistant germplasm materials and the genetic mechanism of heat resistance is unclear. The Chinese rice core germplasm retains the maximum genetic diversity of rice with the minimum colony, and they are effective materials to carry out gene discovery and genetic improvement of rice. The re-sequencing of Chinese rice core germplasm has been completed[9], and based on the heat-resistant phenotype identification of Chinese rice core germplasm, it will be possible to identify heat-resistant genes, so as to provide genetic resources for the genetic improvement of rice's heat resistance. In this study, we perform the heat resistance identification on some Chinese rice core germplasm resources, foreign rice germplasm resources collected and restorer lines during the flowering period, and classify their heat resistance according to setting rate, so as to find heat-resistant rice resources, provide germplasm materials for the improvement of rice's heat resistance, and offer phenotypic data for heat-resistant gene identification.

2 Materials and methods

2.1 Materials The rice materials used include 56 Chinese rice core germplasm, 18 foreign germplasm resources and 6 restorer lines materials, and the heat-resistant control is N22 (Table 1).

2.2 Methods The experimental materials were sown in Nanhu experimental base of Food Crop Research Institute, Hubei Academy of Agricultural Sciences, on May 5 and June 5, 2015, respectively, to ensure that the flowering period of different materials is basically the same. Each experimental material has two treatments (Treatment I: planted in the field as normal temperature control; Treatment II: potted as high temperature treatment). The pot experiment uses 5 L plastic bucket, and the plants of the tested materials are put in the bucket at tillering stage. Each material is planted in 3 buckets, 3 plants for every bucket. At booting stage, 2 – 3 tillers of the same size are kept for each plant, and the excess tillers are cut off. The pots are placed into artificial climate chamber for high temperature stress when the materials in the pots

Received: February 28, 2016 Accepted: April 27, 2016

Supported by Open Project on Food Crop Germplasm Innovation and Genetic Improvement of Hubei Provincial Key Laboratory (2014lzjj02); International Science & Technology Cooperation Program of China (2011DFB31620); Natural Science Foundation of Hubei Province (2014CFA103).

* Corresponding author. E-mail: jiaoch@hotmail.com

begin flowering, and these pots are put out of artificial climate chamber by the end of flowering until maturity. The high temperature treatment period of artificial climate chamber is 9:00—15:00 every day, and the temperature is set at 38℃; in other periods, the temperature is set at 28℃, and the humidity is set at 75%.

2.3 Data measurement and statistical analysis After the materials are mature, the materials under high temperature treatment and in the field are harvested respectively. Then the number of blighted grain and filled grain is measured, and high temperature setting percentage, normal setting percentage and relative spikelet fertility rate are calculated (relative spikelet fertility rate = high temperature setting percentage/normal temperature setting percentage × 100%). SPSS software is used for statistical analysis of data.

Table 1 Name and properties of experimental materials

Experiment No.	Name of materials	Properties of materials	Experiment No.	Name of materials	Properties of materials
15ZG001	Qingke	Core germplasm	15ZG041	Laoguangtou 83	Core germplasm
15ZG002	Babaili	Core germplasm	15ZG042	Zinuo	Core germplasm
15ZG003	Hongqi 5	Core germplasm	15ZG043	Zegu	Core germplasm
15ZG004	Xiushui 115	Core germplasm	15ZG044	Jiefangxian	Core germplasm
15ZG005	Jinyou 1	Core germplasm	15ZG045	Taidongludao	Core germplasm
15ZG006	Chenwan 3	Core germplasm	15ZG046	Dandongludao	Core germplasm
15ZG007	Lamujia	Core germplasm	15ZG047	Lucaihao	Core germplasm
15ZG008	Minbeiwanxian	Core germplasm	15ZG048	Weiguo	Core germplasm
15ZG009	Dongtingwanxian	Core germplasm	15ZG049	Zaoshuxianghei	Core germplasm
15ZG010	Haogedao	Core germplasm	15ZG050	Guangluai 15	Core germplasm
15ZG011	Gu 154	Core germplasm	15ZG051	Maguzi	Core germplasm
15ZG012	Younian	Core germplasm	15ZG052	Yelicanghua	Core germplasm
15ZG013	Zhenxian 232	Core germplasm	15ZG053	Longhuamaomeng	Core germplasm
15ZG014	JWR221	Core germplasm	15ZG054	Jinnante 43B	Core germplasm
15ZG015	9311	Core germplasm	15ZG055	80B	Core germplasm
15ZG016	Yangdao 2	Core germplasm	15ZG056	N22	Core germplasm
15ZG017	Xianggu	Core germplasm	15ZG057	Baxiludao	America
15ZG018	Jixuenuo	Core germplasm	15ZG058	WD-18141	Africa
15ZG019	Xiangwanxian 1	Core germplasm	15ZG059	WD-16210	America
15ZG020	1826	Core germplasm	15ZG060	WD-16376	Oceania
15ZG021	Aimi	Core germplasm	15ZG061	WD-14950	Asia
15ZG022	Xibainian	Core germplasm	15ZG062	WD-14995	Asia
15ZG023	Youmangzaojing	Core germplasm	15ZG063	WD-15446	Asia
15ZG024	Minghui 63	Core germplasm	15ZG064	WD-15610	Asia
15ZG025	Gui 630	Core germplasm	15ZG065	WD-18547	America
15ZG026	Menjiading 2	Core germplasm	15ZG066	WD-16161	America
15ZG027	Benbanggu	Core germplasm	15ZG067	WD-15966	Europe
15ZG028	Heidu 4	Core germplasm	15ZG068	WD-11989	Asia
15ZG029	Xiangdao	Core germplasm	15ZG069	WD-16343	Africa
15ZG030	Aituogu 151	Core germplasm	15ZG070	WD-15887	Asia
15ZG031	Zhenxian 97B	Core germplasm	15ZG071	WD-18362	Oceania
15ZG032	Haobayong 1	Core germplasm	15ZG072	WD-16374	Oceania
15ZG033	Zhonghua 8	Core germplasm	15ZG073	WD-18368	Oceania
15ZG034	Cunsanli	Core germplasm	15ZG074	WD-11661	Asia
15ZG035	Sujing 2	Core germplasm	15ZG075	44064	Restorer lines
15ZG036	Hengxianliangchun	Core germplasm	15ZG076	44076	Restorer lines
15ZG037	Zhongnong 4	Core germplasm	15ZG077	44078	Restorer lines
15ZG038	Putaohuang	Core germplasm	15ZG078	44049	Restorer lines
15ZG039	Lixinjing	Core germplasm	15ZG079	44086	Restorer lines
15ZG040	IR661-1	Core germplasm	15ZG080	44105	Restorer lines

2.4 Heat resistance evaluation standard Based on the evaluation standard of Hu Shengbo *et al.* [10], when the setting percentage under natural conditions ≥ 70% and relative spikelet fertility rate ≥ 70%, it is heat-resistant; when the setting percentage under natural conditions ≥ 70% and relative spikelet fertility rate ≤ 30%, it is sensitive to high temperature; when the setting percentage under natural conditions ≥ 70% and relative spikelet fertility rate = 30% – 70%, it is a medium type.

3 Results and analysis

3.1 Impact of high temperature treatment on rice setting percentage Table 2 shows that the high temperature treatment has a certain impact on the setting percentage of 80 materials for experiment: the number of blighted seed increases and the setting percentage decreases. The high temperature damage in the flowering period has different impact in different materials, and high temperature has the greatest impact on 3 varieties (Haogedao, Benbanggu, Zinuo), and the setting percentage is 0.

Table 2 Impact of high temperature treatment on rice setting percentage Unit: %

No.	Name of materials	Setting percentage			No.	Name of materials	Setting percentage		
		High temperature	Normal temperature	Relative value			High temperature	Normal temperature	Relative value
15ZG001	Qingke	6.1	78.5	7.8	15ZG041	Laoguangtou 83	9.2	78.0	11.8
15ZG002	Babaili	18.0	74.0	24.3	15ZG042	Zinuo	0.0	70.0	0.0
15ZG003	Hongqi 5	37.2	85.9	43.3	15ZG043	Zegu	5.3	83.1	63.7
15ZG004	Xiushui 115	44.5	92.8	47.8	15ZG044	Jiefangxian	7.1	78.2	9.1
15ZG005	Jinyou 1	22.2	64.5	34.4	15ZG045	Taidongludao	8.3	68.9	12.0
15ZG006	Chenwan 3	17.3	83.1	20.8	15ZG046	Dandongludao	13.0	70.0	18.6
15ZG007	Lamujia	25.0	59.0	42.4	15ZG047	Lucaihao	20.0	90.0	22.2
15ZG008	Minbeiwanxian	8.1	88.4	9.2	15ZG048	Weiguo	14.1	80.3	17.6
15ZG009	Dongtingwanxian	3.0	92.0	3.3	15ZG049	Zaoshuxianghei	31.4	87.1	36.1
15ZG010	Haogedao	0.0	56.5	0.0	15ZG050	Guangluai 15	62.5	73.9	84.6
15ZG011	Gu 154	14.2	81.5	17.4	15ZG051	Maguzi	22.1	78.2	28.3
15ZG012	Younian	42.3	90.6	46.7	15ZG052	Yelicanghua	21.0	77.0	27.3
15ZG013	Zhenxian 232	6.0	90.0	6.7	15ZG053	Longhuamaomeng	20.3	84.9	23.9
15ZG014	JWR 221	30.3	81.6	37.1	15ZG054	Jinnante 43B	40.1	80.2	50.0
15ZG015	9311	28.2	90.5	31.2	15ZG055	80B	65.0	80.0	81.3
15ZG016	Yangdao 2	34.2	81.6	41.9	15ZG056	N 22	63.7	85.0	74.9
15ZG017	Xianggu	1.7	80.5	2.1	15ZG057	Baxiludao	8.1	66.2	12.2
15ZG018	Jixuenuo	6.0	69.0	8.7	15ZG058	WD-18141	8.3	80.7	10.3
15ZG019	Xiangwanxian 1	35.2	86.5	40.7	15ZG059	WD-16210	17.3	65.5	26.4
15ZG020	1826	12.0	75.0	16.0	15ZG060	WD-16376	12.0	84.0	14.3
15ZG021	Aimi	18.3	69.8	26.2	15ZG061	WD-14950	56.9	84.5	67.3
15ZG022	Xibainian	5.0	54.0	9.3	15ZG062	WD-14995	6.0	83.0	7.2
15ZG023	Youmangzaojing	34.0	90.0	37.8	15ZG063	WD-15446	5.1	81.3	6.3
15ZG024	Minghui 63	3.2	74.3	4.3	15ZG064	WD-15610	20.1	69.3	29.0
15ZG025	Gui 630	5.1	72.6	7.0	15ZG065	WD-18547	18.2	74.9	24.3
15ZG026	Menjiading 2	10.3	87.5	11.8	15ZG066	WD-16161	23.7	86.0	27.6
15ZG027	Benbanggu	0.0	80.1	0.0	15ZG067	WD-15966	21.2	83.7	25.3
15ZG028	Heidu 4	11.0	81.0	13.6	15ZG068	WD-11989	32.0	76.0	42.1
15ZG029	Xiangdao	3.1	87.5	3.5	15ZG069	WD-16343	71.0	72.1	98.5
15ZG030	Aituogu 151	24.1	60.0	40.2	15ZG070	WD-15887	11.0	57.0	19.3
15ZG031	Zhenxian 97B	2.7	59.0	4.6	15ZG071	WD-18362	22.2	72.5	30.6
15ZG032	Haobayong 1	5.0	67.0	7.5	15ZG072	WD-16374	7.1	78.9	9.0
15ZG033	Zhonghua 8	31.0	86.0	36.0	15ZG073	WD-11661	24.0	87.3	27.5
15ZG034	Cunsanli	3.0	68.0	4.4	15ZG074	WD-18368	6.0	78.0	7.7
15ZG035	Sujing 2	12.1	77.0	15.6	15ZG075	44064	22.9	88.0	26.0
15ZG036	Hengxianliangchun	22.0	82.0	26.8	15ZG076	44076	83.0	92.1	90.1
15ZG037	Zhongnong 4	8.2	82.0	10.0	15ZG077	44078	66.5	90.1	73.8
15ZG038	Putaohuang	22.1	86.0	25.6	15ZG078	44079	81.9	94.5	86.7
15ZG039	Lixinjing	1.0	83.0	1.2	15ZG079	44086	32.0	87.0	36.8
15ZG040	IR 661-1	1.0	74.0	1.4	15ZG080	44105	43.3	86.1	50.3

3.2 Heat resistance of rice materials Most experimental varieties are Chinese rice core germplasm resources or foreign germplasm resources. Due to adaptability of variety, in the 80 experimental materials, 15 have a long growth cycle in the field, and the normal-temperature setting percentage < 70%; the normal-temperature setting percentage of the remaining 66 materials ≥ 70%, in line with the classification criteria of Hu Shengbo[10]. Table 2 shows that for the 65 materials, the relative setting percentage of 41 materials such as Qingke ≤30%, and these 41 materials are sensitive to high temperature; the relative setting percentage of 17 materials such as Zhonghua 8 is 30% to 70%, and these 17 materials are moderately sensitive to high temperature; the relative setting percentage of 7 materials such as N22 (CK), WD - 16343 and 44076 ≥70%, and these 7 materials are resistant to heat.

3.3 Heat-resistant rice materials The experimental material N22 is currently recognized as the heat-resistant rice variety. In this study its relative spikelet fertility rate is 74.9%, and it is the heat-resistant variety, consistent with previous findings. In the 56 Chinese rice core germplasm resources for experiment, it is found that the setting percentage of Guangluai 15 and 80B is slightly vulnerable to high temperature, with relative spikelet fertility rate of 84.6% and 81.3% respectively; for the 18 foreign germplasm materials, the variety WD-16343 from Africa has relative spikelet fertility rate of 98.5%; the restorer lines materials 44076, 44078 and 44079 have high-temperature relative spikelet fertility rate of 90.1%, 73.8% and 86.7%, respectively. 6 new heat-resistant materials are selected, and they are all indica rice varieties.

4 Discussions

Rice germplasm resources are the basic materials for rice variety improvement and gene mining. Through heat resistance identification, 6 new heat-resistant materials are identified from 80 materials, and these materials lay a foundation for the genetic improvement of heat-resistant rice and heat-resistant gene mining. The 3 restorer lines materials are breeding materials with good agronomic traits, and can be directly used as breeding parent. Guangluai 15 and 80B are from Chinese core rice germplasm resources, and their genome sequence information has been very clear[9], so we can easily conduct heat-resistant gene mapping by using them to build genetic groups. For the African resource WD-16343, its relative setting percentage is 98.5%, and among all experimental materials, the high temperature stress has the smallest impact on its setting percentage, showing strong heat resistance. The heat-resistant mechanism of this variety as well as its application value in breeding needs to be further studied. The environmental adaptability of plants is related to their habitat, so does the heat resistance of rice. From the heat resistance of 80 materials, the material with strong heat resistance is mainly indica rice; japonica rice has poor heat resistance, but there are also a handful of materials with strong heat resistance in japonica rice. This rule can provide a reference for us to collect heat-resistant rice germplasm. Reasonable heat resistance identification indicators are the basis of scientific identification of rice's heat resistance. There are no uniform grading standards for heat resistance at present, and the commonly used heat resistance identification indicators include high-temperature setting rate, relative spikelet fertility rate, and high temperature stress index[10-12]. The high-temperature setting percentage directly reflects the maturing rate of rice under high temperature stress, but it can not reflect the differences in setting percentage between rice materials; the relative spikelet fertility and high temperature stress index are relatively reasonable, taking into account high-temperature and normal-temperature setting percentage factors, so they are currently widely used indicators to provide a basis for grading of rice's resistance to high temperature.

References

[1] ZHAO ZW, ZENG ZH, CHEN X, *et al*. Influences of natural high -temperature stress on pollen fertility and main agronomic traits of hybrid rice[J]. Acta Agriculturae Jiangxi, 2009, 21(8): 19 - 21. (in Chinese).
[2] ZHU XM, ZENG QX, NING QL. Effect of natural high temperature on the chasmogamy of hybrid rice[J]. Scientia Agricultura Sinica, 1983(2): 37 - 44. (in Chinese).
[3] MATSUI T, OMASA K, HORIE T. The difference in sterility due to high temperatures during the flowering period among japonica - rice varieties[J]. Plant Prod Science, 2001, 4(2): 90 - 93.
[4] RANG ZW, JAGADISH SVK, ZHOU QM, *et al*. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice[J]. Environmental and Experimental Botany, 2011, 70 (1): 58 - 65.
[5] JAGADISH SVK, CRAUFURD PQ, WHEELER TR. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis[J]. Crop Science, 2008, 48(3): 1140 - 1146.
[6] CHEN QQ, YU SB, LI CH, *et al*. Identification of QTLs for heat tolerance at flowering stage in rice[J]. Scientia Agricultura Sinica, 2008, 41(2): 315 - 321. (in Chinese).
[7] XIAO Y H, PAN Y, LUO L H, *et al*. Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.)[J]. Euphytica, 2011, 178: 331 - 338.
[8] WAN BL, ZHOU YZ, ZHA ZP, *et al*. Tolerance of a restorer line R1056 to high temperature and its application in rice breeding[J]. Agricultural Science & Technology, 2014, 53(4): 753 - 757. (in Chinese).
[9] ZHAO H, YAO W, OUYANG Y, *et al*. RiceVarMap: a comprehensive database of rice genomic variations[J]. Nucleic Acids Research, 2015, 43: D1018 - 1022.
[10] HU SB, ZHANG YP, ZHU DF. Evaluation of heat resistance in hybrid rice[J]. Chinese Journal of Rice Science, 2012, 26(6): 751 - 756. (in Chinese).
[11] FU GF, SONG J, LIAO XY, *et al*. Thermal resistance of common rice maintainer and restorer lines to high temperature stress during flowering and early grain filling stages[J]. Chinese Journal of Rice Science, 2011, 25(5): 495 - 500. (in Chinese).
[12] FANG XW, TANG LH, WANG YP. Selection on rice germplasm tolerant to high temperature[J]. Journal of Plant Genetic Resources, 2006, 7(3): 342 - 344. (in Chinese).