Genetic Variation of Chilling Injury at Seedling Stage in Rice, *Oryza sativa* L.

Tsukasa NAGAMINE and Masahiro NAKAGAHRA

National Institute of Agrobiological Resources, Kannondai, Tsukuba, 305, Japan

To identify an effective method of evaluation of the chilling injury at the seedling stage of rice, 15 combinations of temperature and duration of treatment were examined. Exposure of 12-day-old seedlings to a temperature of five degrees centigrade for 4 days resulted in the widest range of degrees of injury, which produced the optimum conditions for evaluating this character. The reactions to the low temperature indicated wide genetic variations in a total of 2,151 indigenous varieties which originated from various countries. Most of the varieties from high latitudes, Japan, Europe, USA and USSR, were tolerant (Grades 0 to 2), while the varieties from low latitudes, India and Nepal, were predominantly sensitive (Grades 4 to 6). Most of the varieties from Indonesia and the Philippines were relatively tolerant although those countries are located at lower latitudes. Some varieties from Laos, Thailand, Myanmar and Southwest China cultivated in hilly areas were also tolerant. Distinct geographic clines in the variation then occurred along a latitudinal axis. The areas located in Southwest China, Myanmar, Laos, Thailand, Bhutan and Malaysia exhibited a rich diversity in the variations of this character.

KEY WORDS: *Oryza sativa* L., chilling injury, cold tolerance, seedling stage, genetic diversity, geographic cline.

Introduction

Tolerance to low temperature during the germination to the seedling stages is an important character for stable germination and early growth of rice plants and also during the booting and heading stages in the temperate zone and hilly areas. Particularly in the case of direct sowing cultivation practiced in the northern part of Japan, the germination and the early growth of seedlings take place under relatively low temperature conditions. It is thus necessary to improve the tolerance to low temperature stress during those stages. Several varieties of the Indica type are now widely used as mother plants in rice breeding programs to improve the yielding capacity, eating qualities, disease and insect resistance. However, most of these varieties are likely to experience damage by low temperature. Low germinability at low temperature (SASAKI, 1974), damping disease (TAKEICHI *et al.* 1977; NAGAMINE and OZAKI, 1987), low temperature chlorosis (TAKAHASHI and KAIMORI, 1978; CHUONG and OMURA, 1982), low temperature resistance (OKA, 1953), cold tolerance (SASAHARA *et al.* 1982), chilling injury (TAJIMA *et al.* 1983), seedling cold resistance (KWAK *et al.* 1984) have been reported to be associated with low temperature stress during the germination to seedling stages in rice plants and the latter four phenomena seemed to be similar in the plant response. In the present study we designated the withering of seedlings caused by low temperature as chilling injury. Since there is little information about the genetic variation of the chilling injury at the seedling stage, we examined the variation among indigenous varieties. Here we report a method for optimum evaluation of the chilling injury, along with the genetic variation and geographical distribution of the character.

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Materials and Methods

1) Method of evaluation of chilling injury

To identify an effective method of evaluation of the chilling injury, 35 rice varieties were used. Paddy soil used was sterilized by autoclaving at 110°C for 2 hours to prevent the occurrence of damping disease caused by pathogenic fungi. After incubation in a growth chamber at 30°C for 48 hours, 30 seeds of each variety were sown on sterilized soil in a polyethylene pot. Seedlings were grown in a greenhouse at more than 20°C for 12 days to suppress the expression of chlorosis controlled by low temperature chlorosis genes. Low temperature treatments were carried out in a growth chamber after the leaf age became 3.0. The treatments consisted of combination of 5 levels of temperature, 1, 5, 8, 12 and 15°C and 3 periods of exposure, 2, 4 and 7 days. The relative humidity in the chamber was 55–80%, daytime was 6.00 a.m.–8.00 p.m. and the amount of photons was 307 $\mu\text{E}/\text{m}^2\cdot\text{s}$. After the treatments, the seedlings were immediately transferred into another growth chamber under the following conditions: temperature: daytime 26°C - night 22°C, relative humidity: 80%, daytime: 6.00 a.m.–8.00 p.m., amount of photons: 342 $\mu\text{E}/\text{m}^2\cdot\text{s}$. The degree of chilling injury was scored in 7 grades as follows: Grade 0: healthy, Grade 1: tip of leaf blades withered, Grade 2: one third of the area of the second and third leaf blades withered, Grade 3: half of the area withered, Grade 4: two thirds of the area withered, Grade 5: second and third leaf blades withered but leaf sheaths remained green, Grade 6: leaves completely withered (Fig. 1).

2) Materials for evaluating the chilling injury in indigenous rice varieties

A total of 2,151 varieties consisting mostly of indigenous varieties collected from various countries was used to examine the degree of chilling injury. Of these, 71 varieties originated from Japan, 109 from China, 34 from the Philippines, 168 from Indonesia, 81 from Myanmar (Burma), 74 from Thailand, 65 from Laos, 77 from Nepal, 18 from Bhutan, 204 from Malaysia, 986 from India, 20 from Iran and Turkey, 94 from African countries, 89 from European countries, 15 from USSR, 41 from USA and 5 from Brazil. These varieties are genetic stocks preserved at the National Institute of Agrobiological Resources. The temperature condition for the evaluation was 5°C for 4 days in the growth chamber. The degree of injury was determined 6 days after the treatment.

Results

1) Optimum conditions for evaluation of chilling injury

The degree of chilling injury was observed under various conditions including 15 combinations of low temperature and duration of application (Table 1). Of them, 5 treatments, namely 1°C for 2 days, 1°C for 4 days, 5°C for 4 days, 5°C for 7 days and 8°C for 7 days gave very wide variations ranging from Grades 0 to 6 scores. Among them, the treatment at 5°C for 4 days gave the largest variance, resulting in stable scores with high reproducibility. Therefore, such a condition was seemed to optimum evaluation for subsequent genetic analysis.

2) Genetic variation of chilling injury in indigenous rice varieties

Fig. 1. Degree of chilling injury in rice seedlings.

Note: Grade 0: healthy, Grade 1: tip of leaves withered,
 Grade 2: one third of the area in second and third leaves withered,
 Grade 3: half of the area withered, Grade 4: two thirds of the area withered,
 Grade 5: second and third leaves withered but leaf sheaths remained green,
 Grade 6: leaves completely withered.

Table 1. Variation of chilling injury under low temperature conditions using 35 rice varieties¹⁾

Temperature	Combination of low temperature and duration of treatment														
	1°C			5°C			8°C			12°C			15°C		
	2	4	7	2	4	7	2	4	7	2	4	7	2	4	7
Range	0-6	0-6	4-6	0-4	0-6	0-6	0-1	0-4	0-6	0-0	0-1	0-2	0-0	0-0	0-1
Average	2.29	4.13	5.94	0.47	2.45	4.39	0.03	0.34	1.79	0.00	0.03	0.06	0.00	0.00	0.06
Variance	5.15	5.86	0.12	0.67	6.50	5.24	0.03	0.83	6.00	0.00	0.03	0.11	0.00	0.00	0.06
S.D.	2.27	2.42	0.34	0.82	2.55	2.29	0.17	0.91	2.45	0.00	0.17	0.34	0.00	0.00	0.24

1) Numerals show the degree of chilling injury.

A large variation of the chilling injury was observed in the varieties (Table 2). The Japanese indigenous varieties were predominantly tolerant and showed a small variation, namely, 30% of the varieties from Japan gave a Grade 0 score, 47% Grade 1 and 20% Grade 2. Only one variety, Toboshi, which originated from Kagoshima Prefecture and belongs to the Indica type gave a Grade 6 score. Most of the varieties from European countries, particularly from Italy and Hungary, USA and USSR showed a tolerance expressed by Grades 0 to 2. On the other hand, 81% of the Nepalese varieties mainly collected in the Terai area gave Grades 4 to 6 scores, and was sensitive to the chilling injury. While the Indian varieties also gave Grades 3 to 6 scores predominantly. The varieties which originated from high latitudes were generally tolerant, while the varieties from low latitudes were mainly

Table 2. Geographical variations of chilling injury at seedling stage in rice

Geographic origin	Degree of chilling injury ¹⁾							Total
	0	1	2	3	4	5	6	
Japan	21	33	14	2	0	0	1	71
China	22	26	15	15	11	8	12	109
Philippines	2	15	10	5	0	1	1	34
Indonesia	8	36	70	36	5	3	10	168
Myanmar	11	12	6	7	10	8	27	81
Thailand	3	7	19	6	9	8	22	74
Laos	2	7	28	13	6	4	5	65
Nepal	0	2	2	10	12	17	34	77
Bhutan	3	5	2	2	2	1	3	18
Malaysia	18	10	34	23	21	35	63	204
India	11	31	71	149	162	176	386	986
Iran and Turkey	0	8	8	4	0	0	0	20
African countries	1	19	23	25	12	5	9	94
European countries	5	43	29	9	1	1	1	89
USSR	0	6	6	2	1	0	0	15
USA	3	17	13	3	1	2	2	41
Brazil	0	1	2	1	0	1	0	5
Total	110	278	352	312	253	270	576	2,151

- ¹⁾ Grade 0: healthy, Grade 1: tip of leaf blades withered,
 Grade 2: one third of the area in the second and third leaf blades withered,
 Grade 3: half of the area withered, Grade 4: two thirds of the area withered,
 Grade 5: second and third leaf blades withered but the leaf sheaths remained green,
 Grade 6: leaves completely withered, as shown in Fig. 1.

sensitive. Accordingly, a clear altitudinal cline in the variation of the chilling injury was recognized.

The varieties from Southwest China, Myanmar, Laos, Thailand, Bhutan and Malaysia exhibited scores ranging from Grades 0 to 6. The frequency of occurrence of the degree of injury is indicated geographically in Fig. 2 for the varieties from the Asian countries. The varieties from Southwest China, Myanmar, Laos, Thailand, Bhutan and Malaysia exhibited a wide diversity of injury.

Furthermore, the varieties from Indonesia and the Philippines with mainly Grades 1 to 3 scores were relatively tolerant even though these countries are located at low latitudes. Some varieties from the Kashmir region were tolerant with Grades 0 to 2 scores although other Indian varieties collected at an elevation below 1,000m were predominantly sensitive.

Discussion

Although there are several reports on the methods of evaluation of the chilling injury, the methods appeared to require improvement in terms of rapidity of test and accuracy. We investigated a rapid and stable method of evaluation using a growth chamber. The optimum

Fig. 2. Geographical variations of chilling injury in Asian rice varieties.

Note: The vertical axis shows the frequency of occurrence of chilling injury (%).

The horizontal axis shows the degree of chilling injury; 0 (healthy)-6 (leaves completely withered).

conditions for the evaluation of the chilling injury involved a combination of 5°C temperature for a duration of 4 days, which resulted in the widest range of injury degrees and the highest value of variance. TAJIMA *et al.* (1983) adopted a testing method at a temperature of 5-0°C for a period of 5 days using a glasshouse. SALAHUDDIN and VERGARA (1974) and KWAK

et al. (1984) used the screening method in which 10-day-old seedlings are submerged in water at 12°C for 10 days. Compared with the method of SALAHUDDIN and VERGARA (1974) our method enables to evaluate the reaction of the varieties more rapidly. We thus developed an optimum method for the evaluation of the character.

Variations of the chilling injury showed a clear geographical distribution after the screening the 2,151 indigenous rice varieties, indicating a latitudinal cline. The varieties which originated from the northern countries were tolerant to the chilling injury whereas the varieties from southern areas were sensitive. These findings suggested that the indigenous varieties had differentiated to become adapted to their own environmental conditions, particularly to the temperature conditions in the cultivating areas.

The varieties from Southwest China, Myanmar, Laos, Thailand, Bhutan and Malaysia showed a remarkable diversity of the chilling injury. These areas had been already found to correspond to a zone with a wide genetic diversity in characters including esterase isozymes (NAKAGAHRA, 1978), amylose content in endosperm (NAKAGAHRA *et al.* 1986), phenol color reaction (IZUKA *et al.* 1977) and low temperature chlorosis (CHUONG and OMURA, 1982). The present results on the variations of the chilling injury indicated the existence of a wide genetic diversity in those areas in addition to the genetic characteristics described above.

Some varieties from the Kashmir region were tolerant, although most of the Indian varieties were predominantly sensitive. The varieties from Indonesia and the Philippines were relatively tolerant. In addition, some of the varieties from Laos, Thailand, Myanmar and Southwest China cultivated in hilly areas were tolerant. These facts imply that chilling-tolerant varieties can be observed at high altitudes, although the country of origin is located at low latitudes. It is thus concluded that important genetic resources for tolerance to the chilling injury can be found at high latitudes as well as at low latitudes at a high elevation.

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イネ幼植物の低温枯死症状の遺伝変異

長峰 司・中川原捷洋

(農業生物資源研究所, 茨城県つくば市, 〒305)

育苗中に低温に遭遇したイネ幼植物が枯死する低温枯死症状の発生は、育苗の安定化にとって大きな障害であり、この抵抗性は、穂孕期や出穂開花期の低温抵抗性と同様に重要な形質である。栽培稲における低温枯死症状の遺伝変異を明らかにするため、人工気象室を用いた検定方法を検討した。35品種を用いて1, 5, 8, 12, 15℃の処理温度と2, 4, 7日間の処理期間との組合せによる低温処理を行なった。12日苗を用いた場合、1℃・2日間、1℃・4日間、5℃・4日間、5℃・7日間、8℃・7日間処理は反応の品種間変異が大きかったが、このうち、低温に対する反応を最も良く検出できる方法は5℃・4日間処理であった (Table 1)。

アジアを中心に世界各地から収集・導入した2,151品種の在来稲について、5℃・4日間の低温処理に対する反応を処理後6日に調査したところ、地理的変異に明らかな特徴を認めた (Table 2)。日本、ヨーロッパ、米国、ソ連など高緯度原産の品種は低温処理に対して抵抗性のものが多く、一方、インドやネパールの様に低緯度原産の品種は感受性であった。しかし、低緯度であってもインドネシアやフィリピン原産の品種は比較的抵抗性が高かった。インドのカシミール地方の品種のように比較的low緯度であるが、高標高地帯の品種は抵抗性を示した。ラオス、タイ、ミャンマー (旧ビルマ)、中国南西部の一部品種は山岳地帯原産であり、抵抗性であった。中国南西部、ミャンマー、ラオス、タイ、ブータン、マレーシア産の品種は抵抗性から感受性まで幅広い変異を示した (Fig. 2)。

以上のように、低温枯死抵抗性は、高度、緯度すなわち温度環境と対応して明確な地理的傾斜を示している。一方、多様性の中心地域は中国南西部、ミャンマー、ラオス、タイ、ブータン、マレーシアであり、これらの地域はこの形質にとっての遺伝資源の供給地として重要であると考えられる。