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Phenotypic Variation and Selection for Cold-Tolerant Rice (*Oryza sativa* L.) at Germination and Seedling Stages

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Abstract: Owing to its origin in tropical and subtropical areas, rice is susceptible to cold stress. Low temperatures at the germination and seedling stages can result in seed loss, a delayed transplanting period, and lower final yield. In this study, 181 rice varieties from around the world were investigated for cold tolerance at the germination and seedling stages. At the germination stage, the responses of different rice varieties were examined based on the germination index, coleoptile length, and radicle length at low (13 °C) and control temperatures (25 °C). Significant variations in the germination index, coleoptile length, and radicle length were observed among varieties. Low temperature significantly decreased germination ability, and coleoptile and radicle growth in the studied varieties. At the seedling stage, cold tolerance of the rice varieties was evaluated based on the leaf color score under natural low temperature. Similar to the germination stage, at the seedling stage, significant variation in root and shoot growth was observed in the response of rice varieties to low temperature conditions. Based on the results from both the germination and seedling stages, two varieties (Hei-Chiao-Chui-Li-Hsiang and Ta-Mao-Tao) were selected as the best cold-tolerant varieties. Our results also indicate the benefits of warming treatments to protect rice seedlings from low temperature conditions.

Keywords: cold stress; germination stage; rice; screening; seedling stage

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important staple food crops globally and the most important one in Vietnam [1,2]. Although Vietnam is one of the world's major rice exporters, in some mountainous areas, particularly the northwest provinces, it is difficult to maintain food security due to low rice productivity caused by unfavorable environmental conditions. Owing to its origin in tropical and subtropical regions, rice is sensitive to cold stress [3], particularly at the germination and seedling stages [4,5]. In the northern regions of Vietnam, rice cultivation in the spring season (February to June) is usually affected by low temperatures at the germination and seedling stages [6], which results in seed loss and delay in the transplanting period. Breeding and cultivation techniques offer potential ways to overcome these negative effects of cold stress. However, cold tolerance in rice is a complex trait [7].

In northern Vietnam, rice is normally cultivated using the transplanting method. With this method, cold stress at the seedling stage represents one of the most severe abiotic stresses early in the cropping season. However, in recent years, the proportion of direct sowing has been increasing owing to the reduced time and labor required by this method. With the direct sowing method, cold stress can result in severe damage to the sown seeds. To address the problem of cold tolerance, new varieties are required that can resist cold stress at both the germination and seedling stages.

Currently, screening experiments for cold tolerance in rice are generally conducted under controlled conditions, such as in growth chambers. Although screening under controlled conditions provides useful information, it is no substitute for natural conditions. In this study, we performed experiments under both natural and controlled conditions. At the germination stage, screening was performed in a controlled growth chamber. At the seedling stage, rice plants were exposed to a natural condition (low temperature) to elucidate their response to actual conditions encountered during agricultural practice. This study was conducted from 2014 to 2017 at the Faculty of Agriculture, Kyushu University, Japan. Based on the results, we selected specific cold-tolerant varieties for further study. Our results also indicated the beneficial effects of warming treatment on the growth of rice seedlings under cold stress conditions.

2. Materials and Methods

2.1. Plant Materials

Rice genotypes from the Kyushu University Core Collection (KCR), which covers the global distribution of varieties provided by the Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University, Japan, were used in this study. In total, 181 rice varieties were investigated (Tables 1 and 2).

Table 1. Rice varieties used in this study.

KCR Code	Variety Name	Source Country	KCR Code	Variety Name	Source Country
1	C 22	Philippines	58	KUN-MIN-TSIEH-HUNAN	China
2	TAICHUNG NATIVE 1	Taiwan	59	SHAI-KUH	China
3	NHTA10	India	60	SOM CAU 70 A	Vietnam
5	INTAN	Indonesia	62	RTS5	Vietnam
6	TA HUNG KU	China	64	RTS16	Vietnam
7	HEI CHIAO CHUI LI HSIANG KENG	China	65	PEH-KUH-TSAO-TU	Taiwan
9	KEN CHIAO JU HSIAO LI	China	67	EH-IA-CHIU	Taiwan
10	Y CHANG JU	China	70	MACAN BINUNDOK	Philippines
12	CAROLINA GOLD	United States	71	SURJAMKUHI	India
14	KOTOBUKI MOCHI	Japan	73	PADI RAOEKANG	Indonesia
17	CO 25	India	74	SERATOES HARI	Indonesia
18	IGUAPE CATETO	Haiti	75	PADI KASALLE	Indonesia
19	TA-POO-CHO Z	China	76	HU-LO-TAO	China
20	SHORT GRAIN	Thailand	77	PEH-PI-NUO	China
21	KAMENOO	Japan	79	DHOLA AMAN	Bangladesh
22	KIBI	Japan	80	RATHUWEE	Sri Lanka
23	SINTANE DIOFOR	Burkina Faso	81	JC101	India
24	PIN KAEO	Thailand	82	JC111	India
25	DA7	Bangladesh	84	JC149	India
26	DA9	Bangladesh	85	JC157	India
28	DA13	Bangladesh	86	JC178	India
29	PANKHARI 203	India	87	JC1	India
30	DA11	Bangladesh	88	JC73-4	India
31	MAKALIOKA 34	Madagascar	89	TD25	Thailand
32	CHITRAJ(DA 23)	Bangladesh	91	TD 2	Thailand
34	N 22	India	92	JC93	India
35	T 1	India	93	JC92	India
36	N 12	India	94	JC120	India
37	PTB30	India	95	JC117	India
38	CO 18	India	96	JUMALI	Nepal
39	PTB25	India	97	TADUKAN	Philippines

Table 1. *Cont.*

KCR Code	Variety Name	Source Country	KCR Code	Variety Name	Source Country
40	DA8	Bangladesh	98	CHIEM CHANH	Vietnam
41	BASMATI 370	Bangladesh	100	IR5	Philippines
42	TRES MESES	Brazil	102	CUBA 65	Cuba
43	BAMOIA 341	Bangladesh	104	VARY VATO 462	Madagascar
44	BIRAIN 360	Bangladesh	105	ROJOFOTSY 738	Madagascar
46	KARKATI 87	Bangladesh	106	FANDRAPOTSY 104	Madagascar
47	MADAEL	Sri Lanka	107	TSIPALA 421	Madagascar
48	KALUKANTHA	Sri Lanka	108	AVO 742	Madagascar
49	MTU9	India	109	MAINTIMOLOTSY 1226	Madagascar
50	PRATAO	Brazil	110	PAI-KAN-TAO	Taiwan
51	RTS4	Vietnam	111	OS4	West Africa
52	KIANG-CHOU-CHIU	Taiwan	112	NAM SA-GUI 19	Thailand
53	MALAGKIT PIRURUTONG	Philippines	113	MOROBEREKAN	Guinea
54	PACHEHAI PERUMAL	India	114	ARC 7229	India
57	TA-MAO-TAO	China	115	ARC 10177	India

2.2. Evaluation of Cold Tolerance at Germination Stage

2.2.1. Experimental Design and Seed Sowing

The experiment to examine cold tolerance at the germination stage was conducted in growth chambers at the Faculty of Agriculture, Kyushu University, using a randomized complete block design with four replications and 10 seeds/variety/replication.

Seeds were sterilized using 10% ethanol for 3 min and 5% sodium hypochlorite for 30 min, followed by rinsing in distilled water. The seeds were then sown in seedbeds (52.5 × 28 × 4 cm; 8 rows × 16 columns) containing commercial soil (Kokuryu Baido, Seisin Sangyo Co., Kitakyushu, Japan). Water was supplied to the beds to provide suitable moisture for seed germination. The seedbeds were then placed in controlled growth chambers, each with the same photoperiod of 16:00 h light/8:00 h darkness per day. The light intensity inside the growth chambers was 25,000 lx, equivalent to 230 pmol photons m^{-2} s^{-1}. The sown seeds were germinated under two treatments: 21 days at 13 °C (cold stress) and 7 days at 25 °C (control).

2.2.2. Measurement for Parameters

To evaluate the germination ability of the rice varieties under the control and cold stress treatments, the number of germinated seeds was counted daily until 7 days after sowing for the control (25 °C) condition and 21 days after sowing for the cold stress (13 °C) condition. Based on the number of germinated seeds, the germination index (GI%) was calculated using the following formula: GI% = $((G_{14} + G_{21})/10) \times 100$ [8], where G_{14} and G_{21} are the number of germinated seeds at 14 and 21 days, respectively, after sowing under 13 °C, and 10 is the number of seeds per variety counted for each replication.

The coleoptile and radicle length of the germinated seeds were measured at 7 days (at 25 °C, control) and 21 days (at 13 °C, cold stress) after sowing [8]. The percentage decrease in coleoptile and radicle length under cold stress compared to the control condition was calculated based on the cold stress-to-control ratio.

2.3. Evaluation of Cold Tolerance and Warming Treatment at Seedling Stage

2.3.1. Effect of Cold Stress to Leaf Color of Rice Varieties at Seedling Stage

The effect of cold stress at the seedling stage was investigated in a net house at the Faculty of Agriculture, Kyushu University. The experiment was conducted using a randomized complete block design with four replications and four plants/variety/replication. Seeds were sterilized following the method described above for the germination stage, followed by incubation at 30 °C for 72 h for germination. Uniformly germinated seeds of each variety were sown in seedbeds (52.5 × 28 × 4 cm; 8 rows × 16 columns) containing moist sand. The seedbeds were placed on plastic trays (56.5 × 30.0 × 16.7 cm) containing commercial soil (Kokuryu Baido, Seisin Sangyo Co., Kitakyushu, Japan). Seedlings of varieties were nurtured in a net house without plastic cover, and the conditions inside the net house (temperature, humidity) were the same as outside (natural condition). At 3 weeks after sowing time, temperature was decreased to lower 15 °C and remained at this low temperature for 1 month. After 1 month of exposure to low temperature, the leaf color of seedlings was recorded and scored based on the Standard Evaluation System for Rice of the International Rice Research Institute (IRRI) (2002) [9]. This evaluation scale ranges from 1 to 9 based on changes in leaf color, as follows: Seedlings dark green (score = 1; considered the most cold-tolerant genotype); seedlings light green (score = 3); seedlings yellow (5); seedlings brown (7); and seedlings dead (9; considered the most cold-susceptible genotype).

In this experiment, air temperature and air humidity inside the net house were measured by TR-72wf Thermo Recorder (T&D Corporation, Japan). Soil temperature was recorded by TR-71U Thermo Recorder (T&D Corporation, Japan).

Table 2. Rice varieties used in this study (*continued*).

KCR Code	Variety Name	Source Country	KCR Code	Variety Name	Source Country
117	ARC 10352	India	189	TONG-IL	Korea
118	ARC 10497	India	192	FIROOZ	Iran
119	KITRANA 508	Madagascar	193	IR42	Philippines
120	DOM-ZARD	Iran	196	BLACK GORA	India
121	MEHR	Iran	197	PIN TAWNG	Thailand
122	GOMPA 2	India	198	NEP HOA VANG	Vietnam
123	TAOTHABI	India	199	ARC 13829	India
126	63-104	Ivory Coast	200	ARANG	Indonesia
127	THIERNO BANDE	Senegal	201	ARIAS	Indonesia
130	COLOMBIA 1	Colombia	202	CERE AIR	Indonesia
132	IR24	Philippines	205	ILIS AIR	Indonesia
133	KERITING TINGGI	Indonesia	206	PELITA JANGGUT	Indonesia
135	KHAO DAM	Laos	207	POPOT	Indonesia
136	KHAO KAP XANG	Laos	208	TREMBESE	Indonesia
138	MANA MURI	Nepal	209	BADKALAMKATI	India
139	KHAO GAEW	Thailand	211	DA1	India
141	DOURADO PRECOCE	Brazil	213	LAL AMAN	India
145	DHOLI BORO	Bangladesh	214	PATNAI 23	India
147	TEPI BORO	Bangladesh	215	MARAYA	Indonesia
148	DARMALI	Nepal	216	PADI LEBAT	Indonesia
149	KAW LUYOENG	Thailand	217	GOAI	Bangladesh
150	KHAO DAWK MALI 105	Thailand	218	CANELA DE FERRO	Brazil
152	CHAHORA 144	Pakistan	219	DE ABRIL	Brazil
153	JHONA 26	Pakistan	220	LAGEADO	Brazil
155	CHAING RONEA	Cambodia	221	SINNA SITHIRA KALI	Sri Lanka
156	CHAMPATONG	Thailand	222	AI-CHIAO-HONG	China
157	IR29	Philippines	223	PA-TOU-HUNG	China
158	IR30	Philippines	225	BASMATI 217	India
160	RATHAL	Sri Lanka	228	BEONJO	Korea
161	DOMSIAH	Iran	229	CHODONGJI	Korea
166	TCHAMPA	Iran	230	DEOKJEOKJODO	Korea
168	ABRI	Bhutan	231	PATBYEO	Korea
169	FARANGEY	Bhutan	234	CHAU	Vietnam
170	PHUDUGEY	Bhutan	235	BAGHLANI NANGARHAR	Afghanistan
171	GEMJYA JYANAM	Bhutan	236	LUK TAKHAR	Afghanistan
172	RAMINAD STRAIN 3	Philippines	237	MILYANG 55	Korea
173	NP125	India	238	CHHOTE DHAN	Nepal
174	DULAR	India	239	IR56	Philippines
175	KATAKTARA DA 2	Bangladesh	240	IR60	Philippines

Table 2. *Cont.*

KCR Code	Variety Name	Source Country	KCR Code	Variety Name	Source Country
177	PETA	Indonesia	242	BENGIZA	Madagascar
179	TETEP	Vietnam	244	SILAD	Malaysia
180	IR43	Philippines	245	TAN SIBUKU	Malaysia
182	GWA NGASEIN	Myanmar	246	TUMO-TUMO	Malaysia
183	INDANE	Myanmar	248	IR74	Philippines
186	KAUKKYISAW	Myanmar			

2.3.2. Effect of Warming Treatment to Rice Varieties at Seedling Stage

We investigated 50 rice varieties with different leaf color scores to evaluate the effect of warming on rice growth compared to natural conditions (low temperature) at the seedling stage between, March and April 2015. The experiment was conducted following a randomized complete block design with four replications and four plants/variety/replication. The seed sterilization and seed sowing methods were the same as those described above for leaf color screening. During the first week after sowing, seedlings of all varieties were nurtured at 25 °C in a phytotron room. Subsequently, the rice seedlings were transferred to a net house and treated under two different conditions for 2 weeks: Natural low temperature (control) and warming treatment. For the warming treatment, a warming sheet (0.9 × 1.8 m) equipped with an electric thermostat (ND-610, Tukuba Denki Co., LTD., Ibaragi, Japan) was set at 25 °C and placed under the seedling trays.

The shoot length, root length, shoot dry weight, and root dry weight of the different rice varieties were measured. Subsequently, the ratios of these values under the warming treatment to those under the control condition were calculated to evaluate the effect of the warming treatment on the rice varieties compared to the control condition.

2.4. Selection for Cold-Tolerant Varieties

Based on the results of the experiments at both the germination and seedling stages, we selected varieties more tolerant to cold stress conditions.

2.5. Statistical Analysis

Analysis of variance was used to test for differences, followed by Tukey's Honestly Significant Difference test, both using the Statistical Tool for Agricultural Research (STAR) software program (IRRI, Version 2.0.1, Los Baños, Philippines, 2014).

3. Results

3.1. Effects of Cold Stress on Rice Varieties at Germination Stage

3.1.1. Germination Index at Low Temperature

The distribution of the germination index of the studied rice varieties is shown in Figure 1. We observed a marked variation in germination index, ranging from 0 to 95%. Among the 181 varieties studied, 55 varieties (30.4%) had a germination index of 0 (did not germinate under 13 °C), and 46 varieties (25.4%) had a germination index between 0 and 10%. Among 126 germinated varieties, the average germination index was 26.7%, with only 13 varieties having a germination index higher than 50%. Variety KCR 246 (Tumo-tumo) had the highest germination index, 95.0%, followed by KCR 242 (Bengiza, GI = 90.0%).

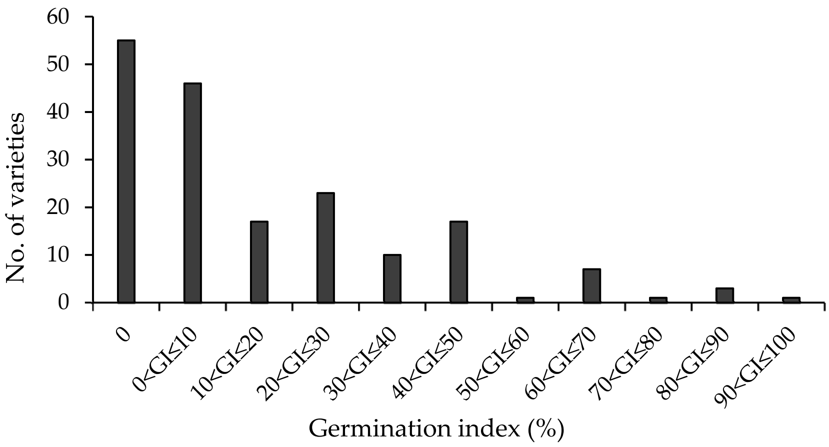

Figure 1. Frequency distribution of germination index of experimental varieties under the cold stress condition.

3.1.2. Coleoptile Length of Rice Varieties

The frequency distribution indicated a large variation in coleoptile lengths of the rice varieties under the control and cold stress conditions (Figure 2A,B). The development of the coleoptile was significantly inhibited under cold stress in comparison to the control condition (Table S1), with the range decreasing from 93.38–99.37% (Table 3). On average, the percentage decrease in the coleoptile length under cold stress, relative to that under the control condition, was 97.72% (Table 3). In comparison to other varieties, KCR 117 (ARC 10352) had the lowest percentage decrease in coleoptile length, 93.38% (Table S1). The coleoptile length of rice varieties under cold stress ranged from 0.33 to 7.0 mm, with the average for all varieties at 2.7 mm. Among all varieties, KCR 57 (Ta-mao-tao) had the longest coleoptile lengths (7.00 mm) under cold stress, followed by KCR 58 (Kun-Min-Tsieh-Hunan, 6.3 mm) and KCR 91 (TD 2, 6.3 mm) (Figure 3).

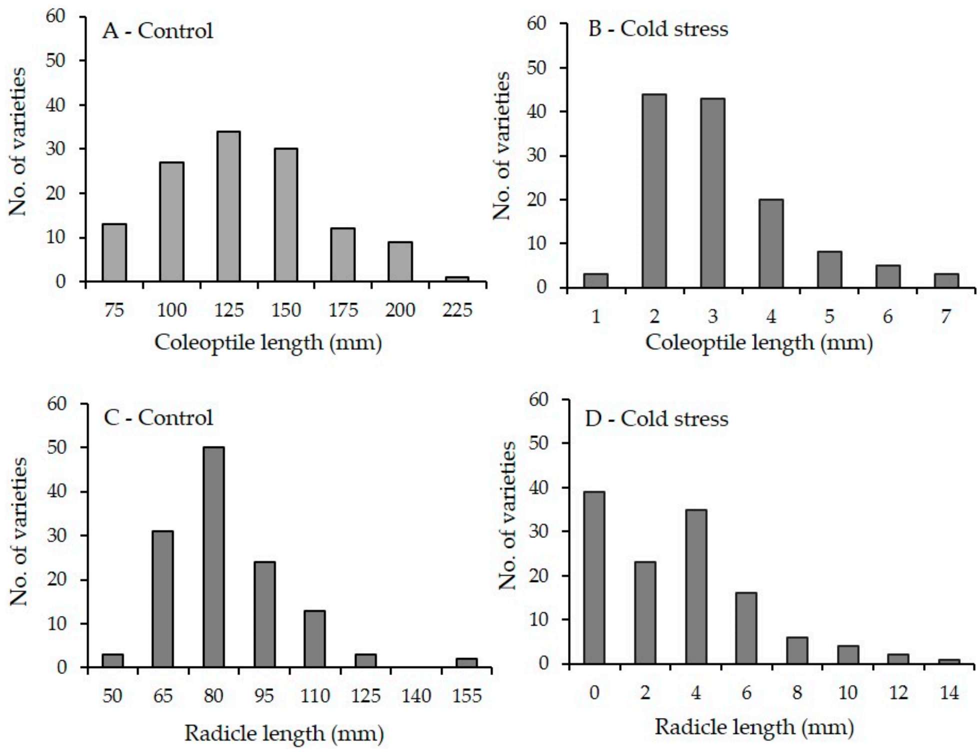

Figure 2. Frequency distribution of coleoptile length (**A**,**B**) and radicle length (**C**,**D**) of rice varieties under the control (25 °C) and cold stress (13 °C) conditions.

3.1.3. Radicle Lengths of Rice Varieties

Figure 2C,D shows the frequency distribution of the radicle length of varieties under the cold stress and control conditions. We observed large variations in the radicle length among the rice varieties under control (with a range from 46.3 to 144.3 mm) and cold stress (with a range from 0.00 to 12.7 mm). KCR 7 (Hei-chiao-chui-li-hsiang-keng) expressed the longest radicle among all varieties (12.7 mm), followed by KCR 77 (Peh-pi-nuo, 10.7 mm) and KCR 84 (JC 149, 10.3 mm) (Figure 3). Similar to the coleoptile, radicle growth was significantly reduced by cold stress compared to the control condition (Table S2), with an average percentage decrease of 96.73% (Table 3). KCR 7 (Hei-chiao-chui-li-hsiang-keng) had the lowest percentage decrease at 81.37%, followed by KCR 84 (JC 149, reduction percentage decrease = 86.45%), and KCR 47 (Madael, percentage decrease = 86.96%) (Table S2).

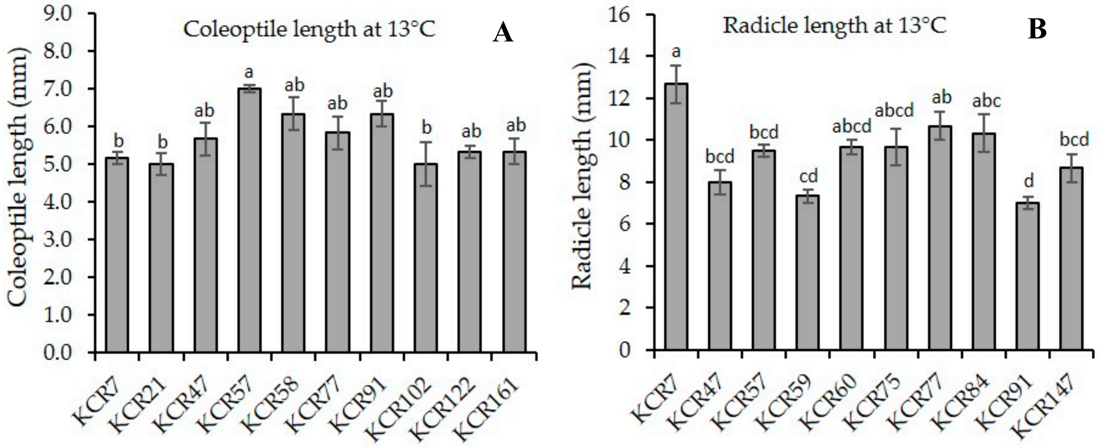

Figure 3. Top ten varieties that had the highest coleoptile length (**A**) and radicle length (**B**) under the cold condition. Means with same letters have no significant difference between varieties ($n = 4$; $p < 0.05$). Values are mean ± SE ($n = 4$).

Table 3. Summary of coleoptile length and radicle length of rice varieties under the control (25 °C) and cold stress (13 °C) conditions.

	Coleoptile Length (mm)			**Radicle Length (mm)**		
	Control	Cold	% Reduction	Control	Cold	% Reduction
Average	118.3	2.7	97.72	76.5	2.5	96.73
Range	50.3–225.0	0.3–7.0	93.38–99.37	46.3–144.3	0.0–12.7	81.37–100.00

3.2. Effects of Cold Stress and Warming Treatment at the Seedling Stage

3.2.1. Environmental Temperature during the Cold Stress Experiment

The environmental conditions, including temperature and humidity, of the experiment are shown in Figure 4. From 1 December 2014 (approximately 3 weeks after sowing), the air and soil temperatures were decreased to below 15 °C and remained at this low temperature for one month, until the time of leaf color score evaluation. We observed no significant difference between the air and soil temperature during the experiment. The relative humidity of the air fluctuated between 40% and 86% during the experiment.

3.2.2. Effects of Cold Stress on Seedling Growth

Among 181 varieties used in this experiment, six varieties showed poor growth and were discarded before the cold stress period. Consequently, 175 rice varieties were evaluated under cold stress conditions at the seedling stage. The response of the rice varieties to cold stress was evaluated based on their leaf color score following the IRRI standard. We observed a marked variation in leaf color score among the studied varieties, with scores ranging from 1 to 9 (Figure 5). After the cold stress period, 30 varieties had a leaf color score of 1 (dark green; defined as the cold-tolerant group, CTG), and 26 varieties had a score of 9 (seedling dead, defined as the cold-sensitive group, CSG) (Figure 5).

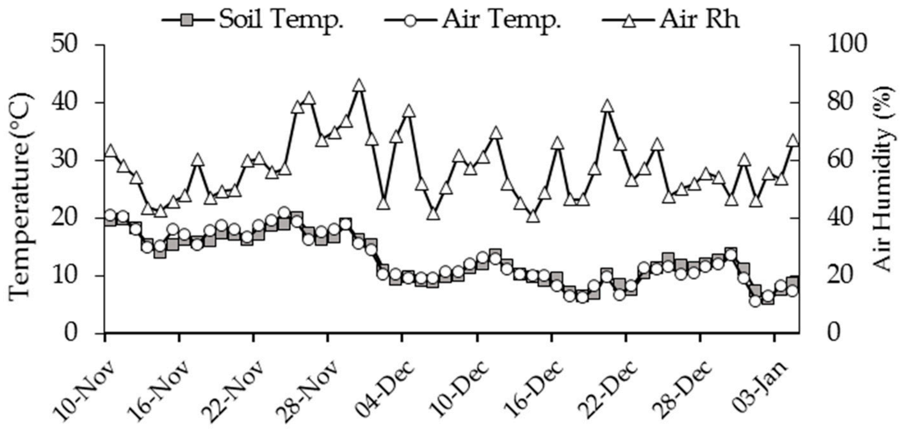

Figure 4. Environmental condition during cold experiment at seedling stage.

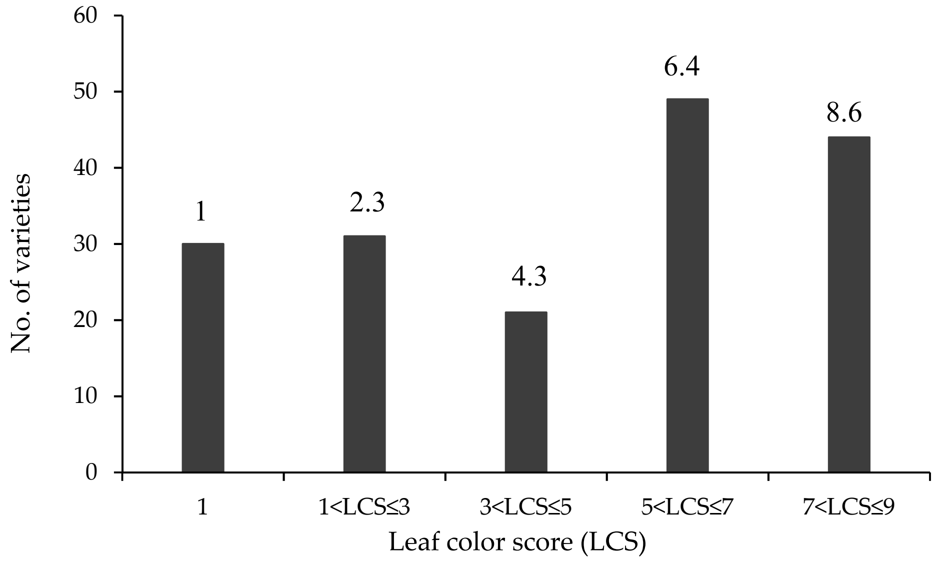

Figure 5. Frequency distribution of leaf color score of varieties under the cold stress condition. Numbers in the top of each column indicate the average leaf color score in each group.

3.2.3. Effect of Warming Treatment on Shoot Growth Compared to Cold Stress

A list of the rice varieties investigated in this experiment is presented in Table 4. The temperature and humidity conditions during the warming treatment period are shown in Figure 6. The soil temperature in the warming treatment generally remained at 5 °C higher than the air and soil temperature under the control condition (cold stress). The results indicate that the warming treatment enhanced shoot growth, increasing the shoot length (average 129.29%) and shoot dry weight (average 142.41%) of the seedlings compared to the control condition (Tables 5 and 6).

Table 4. Rice varieties used in the warming experiment.

KCR Code	Variety Name	Color Score *	KCR Code	Variety Name	Color Score *
7	HEI CHIAO CHUI LI HSIANG KENG	1.0	112	NAM SA-GUI 19	9.0
9	KEN CHIAO JU HSIAO LI	1.0	117	ARC 10352	7.0

Table 4. *Cont.*

KCR Code	Variety Name	Color Score *	KCR Code	Variety Name	Color Score *
17	CO 25	9.0	120	DOM-ZARD	1.0
19	TA-POO-CHOZ	7.7	132	IR24	7.0
21	KAMENOO	1.0	138	MANA MURI	1.0
24	PIN KAEO	7.7	141	DOURADO PRECOCE	1.0
28	DA13	3.7	153	JHONA 26	6.0
29	PANKHARI 203	3.0	155	CHAING RONEA	9.0
34	N 22	5.7	174	DULAR	6.0
41	BASMATI 370	3.7	177	PETA	9.0
50	PRATAO	9.0	178	CARREON	7.0
57	TA-MAO-TAO	1.0	197	PIN TAWNG	9.0
59	SHAI-KUH	1.0	198	NEP HOA VANG	1.0
64	RTS16	7.7	199	ARC 13829	1.0
67	EH-IA-CHIU	1.0	200	ARANG	7.0
70	MACAN BINUNDOK	7.0	205	ILIS AIR	5.5
74	SERATOES HARI	7.7	208	TREMBESE	1.0
80	RATHUWEE	5.7	216	PADI LEBAT	1.0
89	TD25	8.3	219	DE ABRIL	9.0
92	JC93	9.0	220	LAGEADO	9.0
93	JC92	7.0	221	SINNA SITHIRA KALI	5.0
96	JUMALI	2.3	222	AI-CHIAO-HONG	5.0
97	TADUKAN	2.3	223	PA-TOU-HUNG	4.0
101	CUBA 65	9.0	229	CHODONGJI	1.0
105	ROJOFOTSY 738	4.3	237	MILYANG 55	7.0

* Leaf color score of varieties was evaluated under cold stress at seedling stage (Section 3.2.2).

Table 5. Shoot length of varieties under the control (cold) condition and warming treatment.

KCR Code	Control (Cold) (cm)	Warming Treatment (cm)	Warm/ Control (%)	KCR Code	Control (Cold) (cm)	Warming Treatment (cm)	Warm/ Control (%)
7	160.0 ± 8.1	181.3 ± 12.7	113.3 ± 8.8	112	114.0 ± 3.1	192.0 ± 2.3	168.4 ± 6.0
9	140.3 ± 4.8	192.0 ± 14.0	136.8 ± 13.7	117	121.7 ± 1.9	153.7 ± 17.9	126.3 ± 13.0
17	115.3 ± 2.7	148.7 ± 2.9	128.9 ± 3.7	120	198.0 ± 2.0	218.7 ± 0.7	110.4 ± 1.0
19	134.7 ± 4.1	170.0 ± 2.3	126.2 ± 2.1	132	93.7 ± 1.9	112.7 ± 1.8	120.3 ± 4.3
21	166.3 ± 9.8	242.0 ± 14.2	145.5 ± 13.5	138	168.0 ± 9.5	180.7 ± 4.8	107.5 ± 3.1
24	124.7 ± 1.8	177.7 ± 3.2	142.5 ± 2.6	141	137.7 ± 2.8	173.3 ± 15.7	125.9 ± 12.8
28	141.0 ± 3.6	172.7 ± 6.8	122.5 ± 4.3	153	127.3 ± 7.8	156.0 ± 4.2	122.5 ± 4.4
29	143.3 ± 2.4	184.3 ± 2.0	128.6 ± 3.5	155	160.3 ± 14.8	159.0 ± 5.6	99.2 ± 11.0
34	114.7 ± 2.9	142.7 ± 2.7	124.4 ± 1.4	174	116.3 ± 5.8	126.7 ± 2.7	108.9 ± 5.9
41	81.0 ± 27.4	184.7 ± 1.3	228.0 ± 91.6	177	111.3 ± 2.0	142.7 ± 10.9	128.1 ± 7.9
50	147.0 ± 1.0	185.3 ± 38.9	126.1 ± 26.9	178	129.3 ± 9.8	177.0 ± 11.7	136.9 ± 1.5
57	167.0 ± 2.1	210.0 ± 18.9	125.7 ± 11.3	197	166.3 ± 3.3	214.3 ± 11.8	128.9 ± 5.7
59	137.7 ± 2.3	178.0 ± 4.6	129.3 ± 3.9	198	113.0 ± 3.2	148.7 ± 3.5	131.6 ± 2.4
64	126.7 ± 9.4	160.3 ± 5.2	126.6 ± 5.7	199	152.7 ± 11.4	198.7 ± 10.4	130.1 ± 4.3
67	117.0 ± 4.5	178.3 ± 4.9	152.4 ± 9.7	200	116.3 ± 2.3	147.0 ± 4.4	126.4 ± 2.3
70	124.7 ± 5.2	166.7 ± 8.1	133.7 ± 10.8	205	130.3 ± 2.2	148.3 ± 3.8	113.8 ± 4.7
74	130.0 ± 8.1	170.0 ± 5.0	130.8 ± 9.1	208	106.0 ± 2.3	146.7 ± 3.7	138.4 ± 6.5
80	130.3 ± 7.3	184.0 ± 17.5	141.2 ± 18.2	216	172.3 ± 7.8	181.0 ± 2.6	105.0 ± 5.6
89	123.0 ± 3.0	146.3 ± 3.5	119.0 ± 1.8	219	126.7 ± 8.8	165.3 ± 4.1	130.5 ± 10.9
92	108.0 ± 4.6	141.3 ± 4.7	130.9 ± 4.7d	220	142.3 ± 3.8	170.3 ± 4.3	119.7 ± 6.0
93	123.7 ± 4.8	148.7 ± 17.4	120.2 ± 9.8	221	116.0 ± 1.7	140.3 ± 3.9	121.0 ± 4.9
96	139.3 ± 3.7	210.3 ± 14.1	151.0 ± 6.3	222	150.0 ± 3.1	171.7 ± 1.9	114.4 ± 1.6
97	130.0 ± 8.7	194.7 ± 2.9	149.7 ± 8.2	223	160.3 ± 3.5	187.0 ± 8.7	116.6 ± 5.9
101	153.7 ± 3.8	185.3 ± 16.2	120.6 ± 12.5	229	148.3 ± 6.7	188.0 ± 4.2	126.7 ± 3.4
105	151.0 ± 4.9	186.0 ± 5.9	123.2 ± 1.1	237	90.7 ± 6.4	118.0 ± 7.2	130.1 ± 17.3

Table 6. Shoot dry weight of varieties under the control (cold) condition and warming treatment.

KCR Code	Control (Cold) (mg)	Warming Treatment (mg)	Warm to Control Ratio (%)	KCR Code	Control (Cold) (mg)	Warming Treatment (mg)	Warm to Control Ratio (%)
7	22.93 ± 1.0	30.30 ± 1.5	132.12 ± 11.2	112	13.40 ± 2.3	30.33 ± 0.6	226.37 ± 42.0
9	21.70 ± 1.5	31.67 ± 4.4	145.93 ± 10.8	117	16.23 ± 1.3	20.33 ± 3.2	125.26 ± 28.0
17	14.30 ± 1.7	20.57 ± 1.1	143.82 ± 25.1	120	27.07 ± 0.3	26.60 ± 1.7	98.28 ± 5.5
19	18.13 ± 1.2	29.00 ± 3.5	159.93 ± 31.4	132	17.13 ± 0.2	23.60 ± 0.8	137.74 ± 4.8
21	23.00 ± 2.1	38.33 ± 3.9	166.67 ± 23.5	138	22.37 ± 1.1	24.53 ± 3.9	109.69 ± 14.1
24	19.20 ± 0.3	32.23 ± 5.6	167.88 ± 27.2	141	27.53 ± 0.6	31.37 ± 5.3	113.92 ± 19.8
28	15.70 ± 0.8	20.30 ± 1.0	129.30 ± 9.1	153	22.00 ± 2.7	28.67 ± 2.1	130.30 ± 13.5
29	19.17 ± 0.9	28.17 ± 2.5	146.96 ± 8.6	155	19.77 ± 1.1	23.80 ± 1.5	120.40 ± 14.6
34	16.47 ± 0.5	20.13 ± 0.8	122.27 ± 8.1	174	18.30 ± 0.9	23.43 ± 0.4	128.05 ± 7.9
41	16.07 ± 2.3	23.03 ± 0.7	143.36 ± 24.8	177	17.40 ± 3.1	26.37 ± 5.9	151.53 ± 28.8
50	20.60 ± 1.3	40.60 ± 4.1	197.09 ± 10.8	178	21.03 ± 2.3	35.60 ± 2.1	169.26 ± 32.1
57	28.10 ± 0.3	39.87 ± 4.8	141.87 ± 15.8	197	26.00 ± 0.8	33.37 ± 3.2	128.33 ± 12.2
59	21.53 ± 0.9	27.17 ± 3.2	126.16 ± 13.6	198	17.13 ± 1.9	22.43 ± 2.1	130.93 ± 9.0
64	15.83 ± 1.3	21.83 ± 2.8	137.89 ± 10.0	199	24.53 ± 1.9	27.80 ± 2.1	113.32 ± 12.9
67	18.63 ± 0.6	26.10 ± 0.8	140.07 ± 8.9	200	19.87 ± 0.8	25.60 ± 1.0	128.86 ± 3.2
70	18.67 ± 0.5	29.87 ± 2.0	160.00 ± 6.3	205	19.80 ± 1.2	24.10 ± 3.0	121.72 ± 20.5
74	14.83 ± 1.6	18.73 ± 3.2	126.29 ± 30.3	208	19.50 ± 0.9	25.17 ± 1.2	129.06 ± 5.2
80	20.23 ± 1.9	35.17 ± 4.7	173.81 ± 26.7	216	26.00 ± 1.6	32.47 ± 0.8	124.87 ± 9.1
89	24.17 ± 0.3	31.17 ± 1.0	128.97 ± 4.7	219	20.93 ± 3.5	28.40 ± 2.9	135.67 ± 14.5
92	17.83 ± 0.6	30.17 ± 1.1	169.16 ± 11.4	220	22.57 ± 1.0	30.87 ± 0.9	136.78 ± 9.1
93	16.83 ± 1.2	20.70 ± 2.7	122.97 ± 8.5	221	18.00 ± 0.9	28.17 ± 1.7	156.48 ± 8.7
96	26.30 ± 0.8	34.17 ± 3.3	129.91 ± 8.7	222	23.40 ± 0.8	30.53 ± 2.0	130.48 ± 5.9
97	19.43 ± 2.1	30.87 ± 2.2	158.83 ± 18.9	223	23.77 ± 0.5	36.07 ± 2.6	151.75 ± 8.4
101	25.63 ± 1.1	36.57 ± 8.2	142.65 ± 29.8	229	23.03 ± 0.9	28.57 ± 0.4	124.02 ± 5.5
105	25.37 ± 0.2	40.57 ± 6.2	159.92 ± 24.9	237	16.17 ± 2.2	23.47 ± 3.5	145.15 ± 3.8

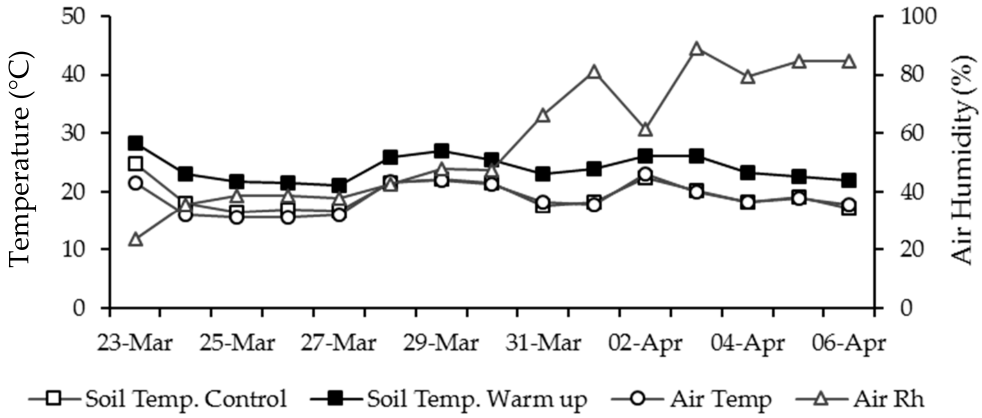

Figure 6. Environmental condition in warming treatment period.

The combination of the warm/control ratio for shoot weight and leaf color score of rice varieties (Figure 7) shows that varieties with lower leaf color score (higher cold tolerance at seedling stage) also expressed the lower warm/control ratio for shoot weight. This suggests that although warming treatment positively affected dry weight accumulation of rice varieties, this method, however, retained a higher effect for cold sensitive varieties than cold tolerant varieties.

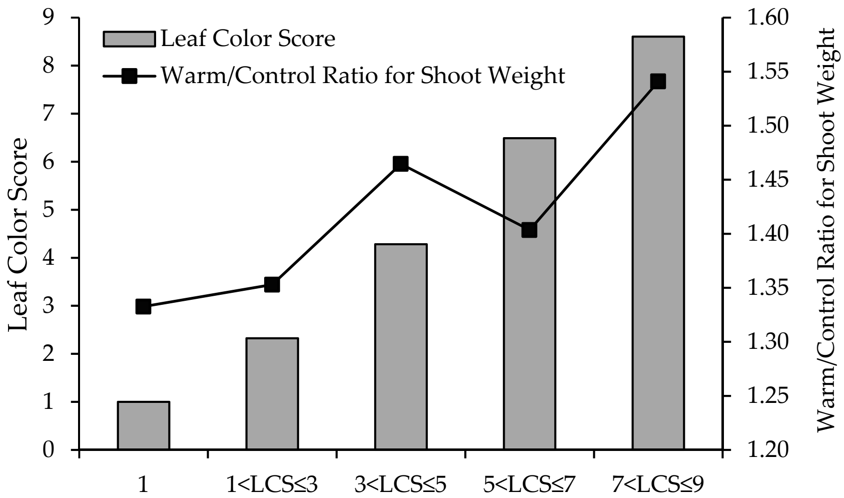

Figure 7. The combination of the average warm/control ratio for shoot weight and leaf color score of rice varieties in the warming treatment experiment.

3.2.4. Effect of Warming Treatment on Root Growth of Rice Varieties

The warming treatment increased root length and root dry weight accumulation, but to a lesser degree than shoot growth (Tables 7 and 8). The average warm/control ratios for root length and root dry weight were 127.83% and 101%, respectively. On average, the CTG had lower warm treatment/control ratios for root length (117.09%) and root weight (86.34%) than did the CSG (warm treatment/control ratios for root length and root weight of 129.01% and 101.78%, respectively).

Table 7. Root length of varieties under the control (cold) condition and warming treatment.

KCR Code	Control (Cold) (cm)	Warming Treatment (cm)	Warm to Control Ratio (%)	KCR Code	Control (Cold) (cm)	Warming Treatment (cm)	Warm to Control Ratio (%)
7	93.3 ± 4.4	126.3 ± 3.8	135.4 ± 8.1	112	81.0 ± 8.6	115.3 ± 12.7	142.4 ± 14.9
9	146.0 ± 4.4	161.7 ± 20.5	110.7 ± 17.6	117	112.0 ± 6.1	125.3 ± 20.7	111.9 ± 13.3
17	100.7 ± 13.8	129.0 ± 17.4	128.1 ± 31.2	120	136.7 ± 13.9	156.3 ± 10.7	114.4 ± 18.9
19	148.0 ± 11.7	103.3 ± 31.8	69.8 ± 17.7	132	107.3 ± 5.5	132.3 ± 8.8	123.3 ± 13.5
21	157.3 ± 9.3	142.7 ± 14.4	90.7 ± 13.2	138	145.0 ± 12.1	140.7 ± 22.3	97.0 ± 15.0
24	98.0 ± 12.5	142.0 ± 17.0	144.9 ± 12.9	141	130.0 ± 10.1	136.0 ± 30.7	104.6 ± 17.2
28	104.7 ± 4.8	90.7 ± 18.1	86.6 ± 19.2	153	96.3 ± 2.9	172.7 ± 12.6	179.2 ± 13.0
29	119.3 ± 9.4	75.3 ± 4.4	63.1 ± 1.7	155	92.0 ± 3.2	180.7 ± 12.7	196.4 ± 20.9
34	110.0 ± 6.4	113.7 ± 12.4	103.3 ± 13.6	174	114.7 ± 5.9	160.7 ± 7.9	140.1 ± 12.7
41	81.3 ± 11.9	108.0 ± 13.1	132.8 ± 30.9	177	108.7 ± 3.2	139.7 ± 13.6	128.5 ± 9.5
50	98.0 ± 6.5	145.7 ± 5.4	148.6 ± 4.9	178	94.7 ± 3.3	184.7 ± 5.9	195.1 ± 8.2
57	116.7 ± 4.8	161.7 ± 17.7	138.6 ± 15.7	197	145.0 ± 13.8	165.7 ± 10.3	114.3 ± 5.5
59	111.3 ± 4.4	201.3 ± 15.4	180.8 ± 20.3	198	120.7 ± 10.7	147.3 ± 16.8	122.1 ± 26.0
64	92.3 ± 5.4	118.0 ± 15.1	127.8 ± 18.8	199	113.7 ± 12.4	114.3 ± 7.2	100.6 ± 14.7
67	120.0 ± 13.8	140.3 ± 17.9	116.9 ± 24.1	200	100.0 ± 3.5	129.3 ± 7.1	129.3 ± 7.2
70	88.7 ± 1.8	176.0 ± 9.5	198.5 ± 13.8	205	113.0 ± 3.5	126.3 ± 16.8	111.8 ± 15.5
74	86.7 ± 4.4	154.0 ± 10.1	177.7 ± 19.4	208	131.7 ± 1.3	150.7 ± 20.7	114.4 ± 16.9
80	114.7 ± 14.7	123.3 ± 2.7	107.6 ± 14.7	216	141.3 ± 4.1	125.3 ± 8.2	88.7 ± 7.4
89	110.7 ± 4.4	145.3 ± 4.4	131.3 ± 3.7	219	106.0 ± 5.0	135.3 ± 21.4	127.7 ± 16.3
92	110.7 ± 19.6	138.7 ± 0.7	125.3 ± 20.3	220	114.7 ± 7.5	169.3 ± 15.6	147.7 ± 14.1
93	108.0 ± 4.5	132.7 ± 8.7	122.8 ± 2.9	221	104.3 ± 4.4	166.7 ± 10.5	159.7 ± 16.5
96	131.0 ± 11.6	142.7 ± 4.4	108.9 ± 7.1	222	121.0 ± 2.7	176.7 ± 12.7	146.0 ± 13.8
97	142.3 ± 17.1	168.7 ± 5.8	118.5 ± 9.9	223	112.7 ± 5.9	165.3 ± 16.9	146.7 ± 14.1
101	147.0 ± 11.6	129.3 ± 10.5	88.0 ± 10.4	229	128.7 ± 8.2	160.0 ± 10.1	124.4 ± 11.9
105	110.0 ± 3.1	147.0 ± 18.0	133.6 ± 12.7	237	103.3 ± 3.5	139.3 ± 5.8	134.8 ± 10.1

Table 8. Root dry weight of varieties under the control (cold) condition and warming treatment.

KCR Code	Control (Cold) (mg)	Warming Treatment (mg)	Warm to Control Ratio (%)	KCR Code	Control (Cold) (mg)	Warming Treatment (mg)	Warm to Control Ratio (%)
7	9.5 ± 1.7	13.8 ± 0.7	145.26 ± 29.7	112	4.9 ± 1.0	7.0 ± 0.7	142.18 ± 34.4
9	12.3 ± 1.5	11.3 ± 1.7	91.62 ± 16.7	117	5.1 ± 0.5	7.3 ± 1.4	143.79 ± 16.1
17	6.5 ± 0.8	5.4 ± 1.3	82.14 ± 22.5	120	11.9 ± 0.7	8.2 ± 2.3	68.82 ± 20.9
19	5.7 ± 0.6	4.0 ± 1.3	70.59 ± 34.5	132	10.9 ± 1.7	10.4 ± 2.0	95.72 ± 18.9
21	11.8 ± 0.4	7.3 ± 1.3	61.97 ± 11.6	138	11.0 ± 1.6	6.7 ± 1.6	60.79 ± 5.6
24	6.1 ± 1.4	7.1 ± 1.6	116.48 ± 41.3	141	9.0 ± 1.6	6.9 ± 2.8	76.30 ± 45.9
28	6.1 ± 0.4	4.7 ± 0.7	76.09 ± 7.5	153	7.1 ± 1.5	9.1 ± 1.5	128.30 ± 7.3
29	6.7 ± 0.4	3.4 ± 0.5	50.75 ± 3.7	155	7.3 ± 1.1	6.6 ± 0.4	90.41 ± 10.1
34	6.3 ± 0.4	4.4 ± 0.1	69.84 ± 5.4	174	6.7 ± 1.2	7.0 ± 0.7	105.00 ± 28.2
41	4.6 ± 1.0	5.1 ± 0.8	112.09 ± 43.8	177	9.1 ± 1.3	8.1 ± 2.7	88.69 ± 45.9
50	6.8 ± 0.7	8.3 ± 1.8	122.55 ± 18.1	178	8.7 ± 1.7	13.8 ± 1.3	159.23 ± 37.4
57	15.7 ± 0.4	15.6 ± 2.9	99.79 ± 21.7	197	8.7 ± 0.8	8.3 ± 1.2	96.15 ± 6.11
59	9.6 ± 1.6	8.9 ± 0.3	93.38 ± 19.4	198	7.1 ± 0.6	6.9 ± 1.7	97.20 ± 27.1
64	6.3 ± 1.0	5.8 ± 1.4	91.53 ± 18.5	199	11.5 ± 2.3	6.6 ± 1.0	57.51 ± 26.1
67	9.1 ± 1.6	10.1 ± 2.0	110.95 ± 46.8	200	11.2 ± 0.8	10.1 ± 1.1	89.61 ± 16.9
70	6.6 ± 1.0	13.2 ± 1.7	198.99 ± 15.3	205	10.0 ± 0.2	6.2 ± 2.6	62.00 ± 27.5
74	3.7 ± 0.8	3.7 ± 0.4	100.00 ± 23.2	208	11.2 ± 2.9	8.0 ± 1.6	71.64 ± 44.5
80	9.4 ± 1.0	8.6 ± 1.2	91.52 ± 8.5	216	12.2 ± 1.6	9.8 ± 1.2	80.60 ± 2.9
89	9.2 ± 1.0	6.4 ± 0.7	69.68 ± 7.6	219	7.0 ± 2.4	8.6 ± 2.0	122.38 ± 62.2
92	5.4 ± 1.3	7.5 ± 0.0	137.42 ± 49.2	220	9.4 ± 0.6	12.2 ± 0.8	129.68 ± 15.1
93	6.8 ± 0.5	4.8 ± 1.2	70.44 ± 22.3	221	6.5 ± 0.4	10.5 ± 0.8	161.86 ± 15.9
96	12.2 ± 1.8	8.3 ± 0.6	67.85 ± 5.8	222	11.7 ± 1.3	8.5 ± 0.4	72.65 ± 4.7
97	7.5 ± 1.0	4.2 ± 0.7	56.44 ± 2.8	223	9.9 ± 1.6	11.6 ± 1.3	117.17 ± 11.9
101	9.7 ± 0.4	5.0 ± 1.3	51.72 ± 15.1	229	10.3 ± 1.3	9.6 ± 1.9	92.90 ± 34.3
105	11.9 ± 2.2	9.8 ± 2.5	81.84 ± 34.6	237	8.7 ± 1.1	7.9 ± 2.4	90.46 ± 15.5

3.3. Selection for Cold-Tolerant Varieties at the Germination and Seedling Stages

Cold-tolerant varieties were selected based on their response to cold stress at both the germination and seedling stages. At the germination stage, KCR 7 had the longest radicle length (12.7 mm) and the eighth-longest coleoptile length (5.2 mm) under cold stress. KCR 57 had the longest coleoptile length (7.0 mm) and the sixth-longest radicle length (9.5 mm) under cold stress (Figure 3). At the seedling stage, the leaf color scores of both KCR 7 and KCR 57 were 1 (Table 4), classified as the highest cold tolerance by the IRRI standard. Based on these results, KCR 7 (Hei-chiao-chui-li-hsiang) and KCR 57 (Ta-mao-tao) were selected as the best cold-tolerant varieties.

4. Discussion

In the north of Vietnam, low temperatures are common during both the germination and seedling stages of rice cultivation. Previous studies have indicated that low temperatures (below 15 °C) severely affect the growth and development of rice during the early growth stages [10,11], and significantly decrease grain yield [12] at harvesting time. In this study, we investigated the responses of 181 rice varieties from around the world to low temperatures. Wang et al. [13] and Farzin et al. [8] reported that the germination ability of rice varieties was depressed under the cold stress condition. Our results confirmed that low temperatures at the germination stage significantly inhibited germination ability (Figure 1), as well as coleoptile (Table 3 and Table S1) and radicle (Table 3 and Table S2) growth, in the studied rice varieties. We observed significant variation in response to cold stress at the germination stage among the studied varieties (Figure 2). Our results are consistent with a previous study by Ñanculao et al. [14]. Shakiba et al. suggested a wide range of variation in germination index among experimental rice varieties [15]. For all varieties, the average percentage decrease in coleoptile length under cold stress compared to the control treatment was 97.72% (Table 3). The average coleoptile length of all varieties under cold stress (13 °C) was 2.7 mm. The radicle length of all varieties was significantly decreased under the cold stress compared to the control condition, with an average percentage decrease of 96.73%. A previous study also reported that cold stress inhibited the elongation and division of cells [16].

The cold tolerance of the rice varieties at the seedling stage was evaluated based on the leaf color score under the natural cold condition (in a net house). In general, low temperature negatively affected the development of the rice varieties at the seedling stage. Among the 175 varieties evaluated at the seedling stage, 26 varieties died under cold stress conditions (with the leaf color score = 9) (Table S3). However, 30 varieties showed no signs of cold damage (leaf color score = 1); we defined these as cold-tolerant varieties (Figure 5 and Table S3). The results suggest a significant variation among the rice populations in response to cold stress at the seedling stage. These results were consistent with a previous study that reported that cold stress inhibited chlorophyll synthesis and chloroplast formation in rice leaves [17]. Donoso et al. [18] also found that cold tolerant genotypes showed lower leaf color scores compared to susceptible ones.

To test the effect of warming treatment on the growth of rice seedlings compared to low temperature, a warming sheet was used to increase the soil temperature to 5 °C higher than the natural temperature. The warming treatment significantly increased the shoot dry weight, shoot length, and root length of the rice varieties compared to these values under the natural condition. However, the effect of the warming treatment differed among varieties. The warming sheet had a more beneficial effect on the cold-sensitive than on the cold-tolerant varieties (Figure 7). These results indicate that the combination of cold-tolerant varieties and warming treatments offers a promising approach to overcoming the negative effects of cold stress during the early growth stages of rice.

5. Conclusions

Breeding for cold-tolerant varieties and using a warming treatment method are promising ways to overcome the negative effects of cold stress on rice during the germination and seedling stages. Germination index and leaf color scores are simple and useful parameters for evaluation of the cold tolerance in rice during germination and seedling stages, respectively. Based on the results of our study at both the germination and seedling stages, two varieties—Hei-Chiao-Chui-Li-Hsiang and Ta-Mao-Tao—were selected as the best cold-tolerant varieties. These varieties are good candidates for further studies on plant breeding for cold stress conditions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/9/8/162/s1, Table S1: Coleoptile length of germinated varieties in germination experiment, Table S2. Radicle length of varieties in germination experiment, Table S3. Leaf color score of varieties under cold temperature at seedling stage.

Author Contributions: D.C.D. performed the experiments, analyzed the data, interpreted the results, and wrote the whole manuscript. T.Y. supervised this research, suggested the data analysis, reviewed the manuscript, and gave valuable comments.

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Conflicts of Interest: We have disclosed that there is no conflict of interest regarding the publication of this article.

References

1. Samuel, S.G. Rice and its importance to human life. In: Biological control of rice diseases. *Prog. Biol. Control* **2009**, *8*, 1–11.
2. Nguyen, H.D.M.; Ellen, J.V.L.; Pieter, R.; Tran, H.T.; Wim, V. Consumer valuation of quality rice attributes in a developing economy. *Br. Food J.* **2018**, *5*, 1059–1072. [CrossRef]
3. Zhang, Q.; Chen, Q.; Wang, S.; Hong, Y.; Wang, Z. Rice and cold stress: Methods for its evaluation and summary of cold tolerance-related quantitative trait loci. *Rice* **2014**, *7*, 24. [CrossRef] [PubMed]
4. Han, L.Z.; Zhang, Y.Y.; Qiao, Y.L.; Cao, G.L.; Zhang, S.Y.; Kim, J.H.; Koh, H.J. Genetic and QTL analysis for low temperature vigor of germinationn in rice. *Acta Genet. Sin.* **2006**, *3*, 998–1006. [CrossRef]

5. Cruz, R.P.; Milach, S.C.K. Cold tolerance at the germination stage of rice: Methods of evaluation and characterization of genotypes. *Sci. Agric.* **2004**, *61*, 1–8. [CrossRef]
6. Doan, V.D.; Nguyen, T.B.; Tran, D.H.; Le, Q.V. *Agricultural Meteorology*; Agricultural Publishing House: Hanoi, Vietnam, 1997; p. 157.
7. Maruyama, K.; Urano, K.; Yoshiwara, K.; Morishita, Y.; Sakurai, N.; Suzuki, H.; Kojima, M.; Sakakibara, H.; Shibata, D.; Saito, K. Integrated analysis of the effects of cold and dehydration on rice metabolites, phytohormones, and gene transcripts. *Plant Physiol.* **2014**, *164*, 1759–1771. [CrossRef] [PubMed]
8. Farzin, P.D.; Mohammad, K.H.; Masoud, E. Methods for rice genotypes cold tolerance evaluation at germination stage. *Int. J. Agric. Crop Sci.* **2013**, *5*, 2111–2116.
9. IRRI (International Rice Research Institute). *Standard Evaluation System for Rice (SES)*; International Rice Research Institute: Los Baños, Philippine, 2002; p. 40.
10. Jiang, L.; Xun, M.M.; Wang, J.L.; Wan, J.M. QTL analysis of cold tolerance at seedling stage in rice (*Oryza sativa* L.) using recombination inbred lines. *Cereal Sci.* **2008**, *48*, 173–179. [CrossRef]
11. Sharifi, P. Evaluation on sixty-eight rice germplasms in cold tolerance at germination. *Rice Sci.* **2000**, *17*, 77–81. [CrossRef]
12. Sava, S. Cold tolerance of temperate and tropical rice varieties. In Proceedings of the 2013 Agribusiness Crop Updates, Crown Perth, Australia, 25–26 February 2013; pp. 1–4.
13. Wang, Z.F.; Wang, J.F.; Wang, F.H.; Bao, Y.M.; Wu, Y.Y.; Zhang, H.S. Genetic control of germination ability under cold stress in rice. *Rice Sci.* **2009**, *16*, 173–180. [CrossRef]
14. Ñanculao, G.D.; Parades, M.; Santos, O.A.; Valásquez, V.B. Cold tolerance evaluation in Chilean rice genotypes at the germination stage. *Chil. J. Agric. Res.* **2013**, *73*, 3–8. [CrossRef]
15. Shakiba, E.; Edwards, J.D.; Jodari, F.; Duke, S.E.; Baldo, A.M.; Korniliev, P.; McCouch, S.R. Genetic architecture of cold tolerance in rice (*Oryza sativa*) determined through high resolution genome-wide analysis. *PLoS ONE* **2017**, *12*, e0172133. [CrossRef] [PubMed]
16. Lyons, J.M. Chilling injury in plants. *Annu. Rev. Plant Physiol.* **1973**, *24*, 445–446. [CrossRef]
17. Sharma, P.; Sharma, N.; Deswal, R. The molecular biology of the low temperature response in plants. *BioEssays* **2005**, *27*, 1048–1059. [CrossRef] [PubMed]
18. Donoso, G.; Cabas, P.; Paredes, M.; Becerra, V.; Balzarini, M. Cold tolerance evaluation of temperate rice (*Oryza sativa* L. ssp. japonica) genotypes at seedling stage. *Gayana Bot.* **2015**, *72*, 1–13. [CrossRef]