九州大学学術情報リポジトリ Kyushu University Institutional Repository

Morphology and Dry Matter Accumulation in Rice (Oryza sativa L.) Seedlings Under Drought Conditions

Dien, Don Cong

Laboratory of Plant Nutrition, Graduate School of Bioresource and Bioenvironmental Sciences, School of Agriculture, Kyushu University | Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Biosciences & Biotechnology, Faculty of Agriculture, Kyushu University

Mochizuki, Toshihiro

Agricultural Ecology Laboratory, Division of Agronomy and Environmental Sciences, Department of Agro-environmental Sciences, Faculty of Agriculture, Kyushu University | Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Biosciences & Biotechnology Faculty of Agriculture, Kyushu University

Yamakawa, Takeo

Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Biosciences & Biotechnology Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/1833811

出版情報:九州大学大学院農学研究院紀要. 62 (2), pp.309-322, 2017-09-08. Faculty of

Agriculture, Kyushu University

バージョン: 権利関係:



Morphology and Dry Matter Accumulation in Rice (*Oryza sativa* L.) Seedlings Under Drought Conditions

Doan Cong DIEN1*, Toshihiro MOCHIZUKI2 and Takeo YAMAKAWA

Laboratory of Plant Nutrition, Division of Molecular Biosciences, Department of Biosciences & Biotechnology, Faculty of Agriculture, Kyushu University, 6–10–1 Hakozaki, Fukuoka 812–8581, Japan (Received April 24, 2017 and accepted May 10, 2017)

Drought is one of the main limitations of rice (*Oryza sativa* L.) productivity and is a severe problem in many regions in the world. In Vietnam, drought affects approximately 22% of the area under rice cultivation and usually occurs during the vegetative stage. One of the difficulties in breeding rice for drought tolerance is the lack of genotypic variation among rice populations. In this study, seedlings of 172 rice varieties from different regions around the world were screened under drought and non-drought conditions in order to explore response to drought stress among different rice genotypes.

Considerable variation in the leaf rolling index (LRI), shoot dry weight, root dry weight (RDW), shoot length (SL), root length (RL), and drought resistance index (DRI) was found between rice varieties over two screening periods. There were positive correlations between DRI and drought to control ratios in RDW, SL, and RL. A negative correlation between DRI and LRI among varieties suggested that LRI might be useful a drought tolerance indicator in rice seedlings. These negative correlations were greater in early afternoon observations than in morning observations. Correlations were also greater in the early stages of drought stress than under severe drought. These results indicate that leaf rolling should be observed in the early afternoon (when drought stress is at its daily maximum) and in the early stages of drought development, before stress becomes severe.

Key words: drought tolerance, rice, screening methods, vegetative stage

INTRODUCTION

Drought is a major problem for rice grown under rainfed lowland and upland conditions, and progress in improving drought resistance through breeding has been slow. A major reason for this slow progress is the complexity of the drought environment, which often results in a lack of clear identification of affected areas. In the mountainous regions of the north and the Red River Delta of Vietnam, droughts frequently occur during the winter-spring season (November-March), i.e., the rice seedling stage. Early-season drought resulting in insufficient standing water in the main paddy makes transplanting difficult; therefore, older seedlings are often used for transplanting. Kumar et al. (2009) reported a positive genetic correlation between yield and early drought stress response, suggesting that genotypes which better respond to drought during early developmental (e.g., seedling) stages might produce greater yield.

Screening rice seedlings for drought tolerance has been performed frequently (Basu *et al.*, 2010; Cui *et al.*, 2008; Gorantla *et al.*, 2007; Kato *et al.*, 2008; Zhou *et al.*, 2006). Such studies require rapid and reliable indicators; a better understanding of drought response mechanisms can help to improve screening protocols. Leaf rolling is a drought avoidance mechanism that reduces water defi-

cit during drought stress (O'Toole and Change, 1978). Loresto et al. (1976), Loresto and Chang (1981) and Chang and Loresto (1986) have suggested leaf rolling as a criterion for scoring drought tolerance in tall and semi-dwarf rice varieties. Varietal difference in leaf rolling or unrolling is correlated with the internal water status of leaf tissues. It is also related to stomatal closure, decreasing transpiration from rice leaves (O'Toole et al., 1979). Diurnal variation is seen in the degree of leaf rolling during water stress, with a maximum at 1:00 P.M. (O'Toole, 1982). Leaf rolling is induced by loss of turgor and poor osmotic adjustment (Hsiao et al., 1984), while delayed leaf rolling is an indication of turgor maintenance and a component of dehydration avoidance (Blum, 1988).

The rate of yield or biomass reduction by stress (e.g., yield under stress as a percentage of yield under nonstress) is a widely used measure of stress resistance in terms of plant production. This ratio requires tests under both stress and fully-irrigated conditions. Fischer and Maurer (1978) improved on this simple ratio by considering the ratio of reduction in one genotype to the mean reduction over all genotypes in a given test. They proposed a drought resistance index (DRI), which is gaining popularity as a useful criterion in selection for drought resistance (e.g., Fukai *et al.*, 1999).

MATERIALS AND METHODS

Materials

A core collection of rice genetic stocks (KCR) covers worldwide distribution varieties provided by Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University was used for this study. The study was

¹ Laboratory of Plant Nutrition, Graduate School of Bioresource and Bioenvironmental Sciences, School of Agriculture, Kyushu University

² Agricultural Ecology Laboratory, Division of Agronomy and Environmental Sciences, Department of Agro–environmental Sciences, Faculty of Agriculture, Kyushu University

^{*} Corresponding author: (E-mail: diendoancong@gmail.com)

divided into two screening periods, with a 1–week interval between screenings. In the first screening, 95 varieties were sown, with 9 varieties discarded before drought treatment due to poor growth. In the second screening, 8 varieties among 94 sown varieties were discarded before drought treatment due to poor germination rates. Consequently, 86 varieties were used for each screening, for a total of 172 varieties (Tables 1 and 2).

Seed sowing and germination

The experiment was conducted in a greenhouse at the Faculty of Agriculture, Kyushu University, in summer 2014. For each screening period, rice seeds were treated with fungicide and dry heat in a forced air oven at $50^{\circ}\mathrm{C}$ for 48 hours, then soaked and germinated at $25^{\circ}\mathrm{C}$ for 4 days. Uniformly germinated seeds of each variety were sown in moist sand seedbeds ($52.5 \times 28 \times 4 \mathrm{~cm}$; 8

Table 1. Rice varieties used in the first screening

KCR code	Variety Name	Source Country	KCR code	Variety Name	Source Country
2	TAICHUNG NATIVE 1	Taiwan	58	KUN-MIN-TSIEH-HUNAN	China
3	NHTA10	India	59	SHAI–KUH	China
5	INTAN	Indonesia	60	SOM CAU 70 A	Vietnam
6	TA HUNG KU	China	62	RTS5	Vietnam
7	Hai CHIAO CHUI LI HSIANG KENG	China	64	RTS16	Vietnam
9	KEN CHIAO JU HSIAO LI	China	65	PEH-KUH-TSAO-TU	Taiwan
10	Y CHANG JU	China	66	HSIA-CHIOH-KEH-TU	Taiwan
14	KOTOBUKI MOCHI	Japan	67	EH–IA–CHIU	Taiwan
17	CO 25	India	70	MACAN BINUNDOK	Philippines
18	IGUAPE CATETO	Haiti	71	SURJAMKUHI	India
19	TA-POO-CHO Z	China	73	PADI RAOEKANG	Indonesia
20	SHORT GRAIN	Thailand	74	SERATOES HARI	Indonesia
21	KAMENOO	Japan	75	PADI KASALLE	Indonesia
22	KIBI	Japan	76	HU–LO–TAO	China
23	SINTANE DIOFOR	Burkina Faso	79	DHOLA AMAN	Bangladesh
24	PIN KAEO	Thailand	80	RATHUWEE	Sri Lanka
26	DA9	Bangladesh	81	JC101	India
28	DA13	Bangladesh	82	JC111	India
29	PANKHARI 203	India	84	JC149	India
30	DA11	Bangladesh	85	JC157	India
31	MAKALIOKA 34	Madagascar	86	JC178	India
32	CHITRAJ(DA 23)	Bangladesh	87	JC1	India
34	N 22	India	88	JC73-4	India
35	T 1	India	89	TD25	Thailand
36	N 12	India	91	TD 2	Thailand
37	PTB30	India	92	JC93	India
38	CO 18	India	93	JC92	India
39	PTB25	India	94	JC120	India
40	DA8	Bangladesh	95	JC117	India
41	BASMATI 370	Bangladesh	96	JUMALI	Nepal
42	TRES MESES	Brazil	97	TADUKAN	Philippines
43	BAMOIA 341	Bangladesh	98	CHIEM CHANH	Vietnam
44	BIRAIN 360	Bangladesh	100	IR5	Philippines
46	KARKATI 87	Bangladesh	101	CUBA 65	Cuba
47	MADAEL	Sri Lanka	104	VARY VATO 462	Madagascar
48	KALUKANTHA	Sri Lanka	105	ROJOFOTSY 738	Madagascar
49	MTU9	India	106	FANDRAPOTSY 104	Madagascar
50	PRATAO	Brazil	107	TSIPALA 421	Madagascar
51	RTS4	Vietnam	108	AVO 742	Madagascar
52	KIANG-CHOU-CHIU	Taiwan	109	MAINTIMOLOTSY 1226	Madagascar
53	MALAGKIT PIRURUTONG	Philippines	110	PAI–KAN–TAO	Taiwan
54	PACHEHAI PERUMAL	India	112	NAM SA–GUI 19	Thailand
57	TA-MAO-TAO	China	178	CARREON	Philippines

Table 2. Rice varieties in the second screening

KCR code	Variety Name	Source Country	KCR code	Variety Name	Source Country
114	ARC 7229	India	179	TETEP	Vietnam
115	ARC 10177	India	180	IR43	Philippines
117	ARC 10352	India	182	GWA NGASEIN	Myanmar
118	ARC 10497	India	183	INDANE	Myanmar
119	KITRANA 508	Madagascar	186	KAUKKYISAW	Myanmar
120	DOM-ZARD	Iran	189	TONG-IL	Korea
121	MEHR	Iran	192	FIROOZ	Iran
122	GOMPA 2	India	193	IR42	Philippines
123	TAOTHABI	India	197	PIN TAWNG	Thailand
126	63–104	Ivory Coast	198	NEP HOA VANG	Vietnam
127	THIERNO BANDE	Senegal	199	ARC 13829	India
129	PATE BLANC MN 1	Ivory Coast	200	ARANG	Indonesia
130	COLOMBIA 1	Colombia	202	CERE AIR	Indonesia
132	IR24	Philippines	205	ILIS AIR	Indonesia
133	KERITING TINGGI	Indonesia	206	PELITA JANGGUT	Indonesia
136	KHAO KAP XANG	Laos	208	TREMBESE	Indonesia
138	MANA MURI	Nepal	209	BADKALAMKATI	India
139	KHAO GAEW	Thailand	211	DA1	India
145	DHOLI BORO	Bangladesh	213	LAL AMAN	India
147	TEPI BORO	Bangladesh	214	PATNAI 23	India
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
157	IR29	Philippines	221	SINNA SITHIRA KALI	Sri Lanka
158	IR30	Philippines	222	AI-CHIAO-HONG	China
160	RATHAL	Sri Lanka	223	PA-TOU-HUNG	China
161	DOMSIAH	Iran	225	BASMATI 217	India
162	GERDEH	Iran	228	BEONJO	Korea
163	GERDEH	Iran	229	CHODONGJI	Korea
164	GHARIB	Iran	230	DEOKJEOKJODO	Korea
165	GHARIB	Iran	234	CHAU	Vietnam
166	TCHAMPA	Iran	236	LUK TAKHAR	Afghanistan
168	ABRI	Bhutan	237	MILYANG 55	Korea
169	FARANGEY	Bhutan	238	CHHOTE DHAN	Nepal
170	PHUDUGEY	Bhutan	239	IR56	Philippines
171	GEMJYA JYANAM	Bhutan	240	IR60	Philippines
172	RAMINAD STRAIN 3	Philippines	242	BENGIZA	Madagascar
173	NP125	India	244	SILAD	Malaysia
174	DULAR	India	245	TAN SIBUKU	Malaysia
175	KATAKTARA DA 2	Bangladesh	246	TUMO-TUMO	Malaysia
177	PETA	Indonesia	248	IR74	Philippines

rows \times 16 columns). Seedbeds were placed on plastic trays (56.5 \times 30.0 \times 16.7 cm) containing commercial soil (Kokuryu Baido, Seisin Sangyo Co., Kitakyushu, Japan). Until drought treatment began, soil in all trays was fully saturated.

Drought treatment

Drought treatment began one week after sowing day by withdrawing water; the water level in control units was maintained at 1 cm above the soil surface. Drought treatment was continued until every variety exhibited permanent wilting, then water was re–supplied to promote recovery.

Measurements

From the first day of drought treatment, the leaf rolling index (LRI) of each variety was observed twice per day, in the morning (7:00 A.M) and early afternoon (1:00 P.M), based on the IRRI standard (Table 3). Permanent wilting time was defined as the time when all varieties reached an LRI of 9 during the morning observation period, from which they did not recover the following morning.

Plant height and number of tillers were observed on the permanent wilting day and seven days after watering resumed.

Table 3. Standard for leaf rolling index at vegetative stage (IRRI, 2002)

Score	Leaves morphology
0	Leaves healthy
1	Leaves start to fold (shallow)
3	Leaves folding (deep V-shape)
5	Leaves fully cupped (U-shape)
7	Leaves margins touching (0-Shape)
9	Leaves tightly rolled

Plant samples were collected seven days after watering resumed to record root length and root and shoot dry biomass. The drought resistance index (DRI) of each variety was calculated as:

Drought Resistance Index (DRI)

where Biomassdrought and Biomasscontrol are the biomass of the sample under drought and control conditions, respectively; and Meandrought and Meancontrol are the mean biomass of all samples under drought and control conditions, respectively, following Fischer and Maurer (1978).

RESULTS

Leaf rolling index (LRI)

Tables 4 and 5 show LRI values for rice varieties from the first and second screenings, respectively. There was considerable variation in LRI values among genotypes in both the morning and afternoon of the first screening, on all days. Leaf rolling began in the afternoon and afternoon LRI values were always greater than morning values on the same day.

We began to record LRI in the afternoon 12 days after drought treatment began (12 DAD). The average LRI value at this time was 3.62, ranging from 0.00 (KCR 86, KCR 88, and KCR 93) to 7.00 (KCR 5). On the IRRI LRI scale, a value of 0.00 indicates that leaves were completely healthy, with no evidence of leaf rolling, while a value of 7.00 indicates strongly rolled leaves, with two leaf edges touching together. LRI continued to rise in all

varieties as drought treatment continued. The average LRI values recorded in the afternoon were 4.65 at 14 DAD, 5.69 at 15 DAD, and 7.72 at 16 DAD. At 17 DAD, afternoon LRI values of all varieties reached a maximum (9.0).

Morning LRI values increased as drought treatment continued, and were very different between varieties. Average morning LRI values increased from 4.90 at 15 DAD to 6.24 at 17 DAD, and reached 8.96 at 19 DAD. In general, there was greater variation between genotypes in morning LRI values than in afternoon LRI values (Table 4a, b).

A similar trend occurred during the second screening period, when there was significant variation in both morning and afternoon LRI values (Table 5 a, b). The average LRI for 12 DAD was 1.74, with values ranging from 0.00 (KCR 114, KCR 122, KCR 157, KCR 158, KCR 160, KCR 162, and KCR 165) to 7.00 (KCR 126). The average afternoon LRI values of LRI were 5.41 at 14 DAD, 7.79 at 17 DAD and 8.50 at 17 DAD. There was a large variation in morning LRI values, which increased as drought treatment continued. The average morning LRI values at 15 DAD, 17 DAD, and 19 DAD were 2.82, 7.07, and 8.18 respectively.

Dry matter accumulation and morphology

During both screening periods, we saw evidence that drought treatment had reduced dry matter accumulation in shoots and roots, and reduced shoot length (SL) and root length (RL) (Tables 6 and 7). There were considerable genotypic variations in SDW, RDW, SL and RL and all traits showed normal frequency distribution in both screening periods (Fig. 1, 2, 3 and 4).

During the first screening, the average shoot dry weights (SDW) for control and drought seedlings were 774 and 260 mg, respectively. On average, the drought/control ratio of SDW was 0.33 for all varieties. There was a large range in SDW for control seedlings (313–1397 mg) and drought seedlings (70–517 mg) (Table 6). The maximum SDW for the control treatment was 1397 mg (KCR 108), and the maximum SDW for the drought treatment was 517 mg (KCR 178). The average drought/control ratio of RDW was 0.48, ranging from 0.17 (KCR 101) to 0.90 (KCR 85 and KCR 112). The drought/control ratios for SL and RL in the first screening were 0.65 and 0.83, respectively (Table 8).

Dry matter accumulation and morphology also varied greatly in the second screening for both drought and control treatments. SDW in the control treatment ranged from 257 (KCR 127) to 2147 mg (KCR 123), and in the drought treatment from 40 (KCR 126) to 357 mg (KCR 205). The drought/control ratio of SDW in the second screening was much lower (0.18) than in the first screening (0.33). The drought/control ratios of RDW and SL were also lower than those in the first screening. The drought/control ratio of RL (0.88) was higher than the ratio of RDW (0.26) and SL (0.51) (Table 8).

Drought resistance index

There was considerable variation in DRI between

 $\textbf{Table 4. a.} \ \ \text{Leaf rolling index of varieties in the first screening}$

KCR		Early afternoon	n observation		Mor	ning observation	
code	12 DAD	14 DAD	15 DAD	16 DAD	15 DAD	17 DAD	19 DAD
2	4.00 ± 0.58	4.50 ± 0.50	6.00 ± 0.58	7.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	9.00
3	5.00 ± 0.00	5.50 ± 0.50	6.50 ± 1.11	8.00 ± 0.58	6.00 ± 0.58	6.50 ± 1.00	9.00
5	7.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.58	8.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00	9.00
6	5.50 ± 0.96	5.50 ± 0.96	7.50 ± 0.96	8.50 ± 0.50	6.50 ± 1.11	7.50 ± 1.91	9.00
7	5.00 ± 1.15	5.00 ± 1.15	6.50 ± 0.50	7.50 ± 0.50	6.50 ± 0.58	7.50 ± 1.91	9.00
9	4.00 ± 0.58	4.00 ± 0.58	5.50 ± 0.50	7.50 ± 0.50	5.00 ± 0.00	5.50 ± 1.91	9.00
10	3.00 ± 0.00	3.67 ± 0.58	5.00 ± 0.00	7.67 ± 0.58	4.33 ± 0.58	7.00 ± 0.00	9.00
14	3.33 ± 1.44	4.33 ± 0.58	5.00 ± 1.15	7.00 ± 1.00	4.33 ± 0.58	5.67 ± 2.31	9.00
17	5.00 ± 0.82	5.50 ± 0.96	6.50 ± 0.58	8.00 ± 0.58	5.50 ± 0.96	6.50 ± 1.00	9.00
18	4.00 ± 1.80	4.25 ± 1.49	6.00 ± 1.00	7.00 ± 1.00	5.67 ± 1.33	5.67 ± 2.31	9.00
19	6.50 ± 0.50	6.50 ± 0.50	7.00 ± 0.82	9.00 ± 0.00	5.50 ± 0.58	7.00 ± 1.63	9.00
20	3.67 ± 0.58	4.33 ± 0.58	5.00 ± 0.00	7.00 ± 0.00	5.00 ± 0.00	6.33 ± 1.15	9.00
21	6.50 ± 0.50	6.50 ± 0.50	6.50 ± 0.50	9.00 ± 0.00	6.50 ± 0.58	8.50 ± 1.00	9.00
22	3.50 ± 0.50	3.50 ± 0.50	5.50 ± 0.50	7.00 ± 0.00	5.00 ± 0.94	5.50 ± 1.91	9.00
23	3.00 ± 0.00	4.33 ± 0.58	5.67 ± 0.67	7.00 ± 0.00	4.33 ± 0.58	6.33 ± 1.15	9.00
24	5.00 ± 0.00	5.00 ± 0.00	6.00 ± 0.58	7.67 ± 0.58	5.50 ± 0.58	5.67 ± 1.15	9.00
26	4.50 ± 0.50	5.50 ± 0.50	6.50 ± 0.58	9.00 ± 0.00	6.00 ± 0.58	7.00 ± 0.00	9.00
28	4.50 ± 0.50	6.00 ± 0.58	7.50 ± 0.96	9.00 ± 0.00	6.50 ± 0.58	8.00 ± 1.15	9.00
29	3.00 ± 0.00	4.50 ± 0.50	6.50 ± 0.58	8.50 ± 0.50	5.00 ± 0.82	5.50 ± 1.91	9.00
30	4.33 ± 0.58	5.00 ± 0.00	6.33 ± 0.67	8.33 ± 0.58	5.00 ± 0.00	7.00 ± 0.00	9.00
31	6.00 ± 0.58	6.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00	6.50 ± 0.50	7.00 ± 0.00	9.00
32	6.00 ± 0.58	6.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00	6.50 ± 0.50	7.00 ± 0.00	9.00
34	4.00 ± 0.50	6.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00	7.00 ± 0.00	7.00 ± 1.63	9.00
35	6.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00	9.00 ± 0.00	8.50 ± 0.58	9.00 ± 0.00	9.00
36	3.50 ± 0.50	4.50 ± 0.50	6.50 ± 0.58	7.00 ± 0.82	6.00 ± 1.00	8.00 ± 2.00	9.00
37	6.50 ± 0.50	8.00 ± 0.58	8.00 ± 0.58	8.50 ± 0.50	7.50 ± 0.67	7.50 ± 1.00	9.00
38	2.25 ± 0.75	4.33 ± 0.58	5.67 ± 0.67	6.33 ± 0.58	4.33 ± 0.58	6.33 ± 1.15	9.00
39	3.00 ± 0.00	5.50 ± 0.50	6.50 ± 0.50	8.50 ± 0.50	5.00 ± 0.94	7.00 ± 0.00	9.00
40	1.00 ± 0.87	2.00 ± 0.87	3.00 ± 0.00	7.67 ± 0.58	2.33 ± 1.33	2.33 ± 2.31	9.00
41	2.25 ± 0.75	2.25 ± 0.75	4.00 ± 0.67	7.00 ± 0.00	2.50 ± 0.50	4.50 ± 1.00	$8.33 \pm 1.$
42	2.00 ± 0.87	3.00 ± 0.00	4.33 ± 0.67	6.33 ± 0.58	3.67 ± 0.58	4.33 ± 1.15	9.00
43	2.25 ± 0.75	3.50 ± 0.50	5.00 ± 0.00	9.00 ± 0.00	5.00 ± 0.94	7.00 ± 0.00	$8.33 \pm 1.$
44	5.67 ± 0.58	5.67 ± 0.58	7.00 ± 0.00	9.00 ± 0.00	5.67 ± 0.58	7.00 ± 0.00	9.00
46	5.50 ± 0.96	4.50 ± 0.50	6.00 ± 0.67	9.00 ± 0.00	5.00 ± 0.00	7.50 ± 1.00	9.00
47	3.50 ± 0.50	5.50 ± 0.50	5.50 ± 0.50	8.50 ± 0.50	5.50 ± 0.58	6.00 ± 1.15	9.00
18	6.50 ± 0.50	7.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00	7.00 ± 0.00	8.00 ± 1.15	9.00
19	3.00 ± 0.00	5.50 ± 0.96	5.50 ± 1.11	8.00 ± 0.58	5.00 ± 0.82	5.50 ± 1.91	9.00
50	6.50 ± 0.50	8.00 ± 0.58	8.00 ± 0.58	8.00 ± 0.58	7.00 ± 0.00	7.50 ± 1.00	9.00
51	3.50 ± 0.50	6.00 ± 0.58	6.50 ± 0.96	7.00 ± 0.82	5.67 ± 0.67	5.67 ± 1.15	9.00
52	4.00 ± 0.58	7.00 ± 1.15	8.00 ± 0.58	7.50 ± 0.50	6.50 ± 0.58	7.50 ± 1.00	9.00
53	6.00 ± 1.00	7.00 ± 0.82	8.50 ± 0.58	9.00 ± 0.00	8.00 ± 0.58	9.00 ± 0.00	9.00
54	6.00 ± 0.58	6.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00	6.50 ± 0.58	8.50 ± 1.00	9.00
57	3.50 ± 0.50	5.50 ± 0.50	6.00 ± 0.67	8.50 ± 0.50	5.50 ± 0.96	7.00 ± 1.63	9.00
58	4.50 ± 0.96	6.50 ± 0.50	7.50 ± 0.50	9.00 ± 0.00	7.00 ± 0.00	8.00 ± 1.15	9.00

 $\textbf{Table 4. b.} \ \ \text{Leaf rolling index of varieties in the first screening } (\textit{continued})$

KCR		Early afternoon	n observation	Morning observation				
code	12 DAD	14 DAD	15 DAD	16 DAD	15 DAD	17 DAD	19 DAD	
59	5.00 ± 1.15	6.00 ± 1.00	6.50 ± 0.58	8.50 ± 0.50	6.00 ± 1.00	6.50 ± 1.00	9.00	
60	5.50 ± 0.96	6.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00	9.00	
62	4.33 ± 1.15	5.67 ± 0.58	7.00 ± 0.00 7.00 ± 0.00	9.00 ± 0.00 9.00 ± 0.00	6.33 ± 0.58	7.67 ± 1.15	9.00	
64	4.93 ± 1.13 5.00 ± 1.00	7.00 ± 1.00	7.00 ± 0.00 7.00 ± 0.00	9.00 ± 0.00 9.00 ± 0.00	7.00 ± 1.15	9.00 ± 0.00	9.00	
65	3.50 ± 0.50	4.50 ± 0.50	6.00 ± 0.00 6.00 ± 0.00	9.50 ± 0.50 8.50 ± 0.50	7.00 ± 1.13 5.50 ± 0.50	9.00 ± 0.00 8.50 ± 1.00	9.00	
		4.50 ± 0.50 7.50 ± 0.50						
66	5.50 ± 0.50		7.50 ± 0.50	9.00 ± 0.00	6.50 ± 0.67	9.00 ± 0.00	9.00	
67	4.00 ± 1.00	5.00 ± 0.8	5.50 ± 1.26	8.50 ± 0.50	5.50 ± 1.45	8.00 ± 2.00	9.00	
70	6.00 ± 0.58	6.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00	6.00 ± 0.67	9.00 ± 0.00	9.00	
71	3.50 ± 0.50	5.00 ± 1.15	7.00 ± 0.94	9.00 ± 0.00	6.00 ± 1.00	8.50 ± 1.00	9.00	
73	2.25 ± 0.75	5.00 ± 0.00	6.00 ± 0.67	7.00 ± 0.00	5.50 ± 0.50	7.00 ± 0.00	9.00	
74	3.50 ± 0.50	4.50 ± 0.50	6.50 ± 0.58	9.00 ± 0.00	4.50 ± 0.50	8.50 ± 1.00	9.00	
75	4.50 ± 0.50	4.50 ± 0.95	5.50 ± 0.58	9.00 ± 0.00	4.50 ± 0.50	6.50 ± 1.00	9.00	
76	2.25 ± 0.75	3.50 ± 0.96	6.00 ± 0.67	8.50 ± 0.50	5.00 ± 1.15	6.00 ± 1.15	9.00	
79	3.00 ± 0.00	3.50 ± 0.50	5.00 ± 0.00	9.00 ± 0.00	3.50 ± 0.50	6.00 ± 1.15	9.00	
80	1.50 ± 0.87	3.50 ± 0.50	4.50 ± 0.58	8.00 ± 0.58	3.50 ± 0.50	6.00 ± 1.15	9.00	
81	1.50 ± 0.87	2.75 ± 1.03	3.00 ± 0.82	6.00 ± 0.58	1.00 ± 0.00	1.00 ± 0.00	8.00 ± 2.00	
82	2.25 ± 0.75	3.00 ± 0.00	4.00 ± 0.67	5.50 ± 0.50	3.00 ± 0.00	1.50 ± 1.00	9.00	
84	3.00 ± 0.00	4.00 ± 0.58	4.00 ± 0.58	5.50 ± 0.50	3.50 ± 0.58	3.00 ± 1.63	9.00	
85	3.50 ± 0.50	3.50 ± 0.50	4.50 ± 0.50	8.00 ± 0.58	4.00 ± 0.67	4.50 ± 1.91	9.00	
86	0.00 ± 0.00	1.00 ± 0.0	0.00 ± 0.00	2.50 ± 0.50	1.00 ± 0.00	1.00 ± 0.00	7.50 ± 1.00	
87	2.25 ± 0.75	4.00 ± 0.58	4.50 ± 0.58	7.00 ± 0.00	4.00 ± 0.58	6.50 ± 1.00	9.00	
88	0.00 ± 0.00	0.75 ± 0.25	1.50 ± 0.50	5.50 ± 0.96	1.50 ± 1.00	2.50 ± 1.91	9.00	
89	3.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	9.00	
91	3.67 ± 0.58	5.00 ± 0.00	5.67 ± 0.67	7.00 ± 0.00	5.00 ± 0.00	5.67 ± 1.15	9.00	
92	2.25 ± 0.75	4.50 ± 050	5.50 ± 0.58	8.50 ± 0.50	5.00 ± 0.82	7.00 ± 0.00	9.00	
93	0.00 ± 0.00	1.25 ± 0.63	3.75 ± 1.44	6.50 ± 0.50	1.50 ± 0.50	5.00 ± 2.83	9.00	
94	0.25 ± 0.25	0.50 ± 0.29	4.50 ± 0.58	6.50 ± 0.50	1.00 ± 0.71	7.00 ± 0.00	9.00	
95	1.50 ± 0.87	4.00 ± 0.58	5.00 ± 0.94	6.00 ± 0.58	4.00 ± 0.58	5.00 ± 2.31	9.00	
96	1.50 ± 0.87	3.50 ± 0.50	4.50 ± 0.58	6.50 ± 0.50	3.50 ± 0.50	5.00 ± 2.31	9.00	
97	2.25 ± 0.75	2.25 ± 0.75	4.00 ± 0.67	7.00 ± 0.00	2.50 ± 0.50	4.50 ± 1.00	9.00	
98	3.00 ± 0.00	4.50 ± 0.50	5.50 ± 0.58	8.00 ± 0.58	4.50 ± 0.50	6.50 ± 1.00	9.00	
100	1.50 ± 0.87	3.50 ± 0.50	4.00 ± 0.67	7.00 ± 0.00	3.50 ± 0.50	6.50 ± 1.10	9.00	
101	4.50 ± 0.50	4.50 ± 0.96	4.50 ± 0.96	8.00 ± 0.58	4.50 ± 0.58	6.00 ± 1.15	9.00	
104	2.00 ± 0.87	2.00 ± 0.87	3.00 ± 1.15	7.67 ± 0.58	2.33 ± 0.58	3.67 ± 1.15	9.00	
105	2.00 ± 0.87	3.00 ± 0.00	3.67 ± 0.58	7.00 ± 0.00	3.67 ± 0.67	3.67 ± 1.15	9.00	
106	2.25 ± 0.75	3.00 ± 0.00	4.00 ± 0.67	7.00 ± 0.82	3.50 ± 0.50	4.00 ± 1.15	9.00	
107	2.25 ± 0.75	4.00 ± 0.58	4.50 ± 0.50	5.50 ± 0.50	3.50 ± 0.58	4.00 ± 1.15	9.00	
108	1.50 ± 0.87	2.25 ± 0.75	3.50 ± 0.58	5.50 ± 0.50	2.50 ± 0.50	3.50 ± 3.00	9.00	
109	1.00 ± 0.87 1.00 ± 0.87	3.00 ± 0.00	5.00 ± 0.00	7.67 ± 0.58	3.67 ± 0.58	5.00 ± 5.00	9.00	
110	5.00 ± 0.00	5.00 ± 0.00 5.00 ± 0.00	6.00 ± 0.00 6.00 ± 1.00	7.00 ± 0.82	5.00 ± 0.94	6.00 ± 9.00 6.00 ± 2.00	9.00	
112	3.00 ± 0.00 3.00 ± 0.00	3.00 ± 0.00 3.00 ± 0.00	4.33 ± 0.67	5.67 ± 0.58	3.67 ± 0.58	5.00 ± 0.00 5.00 ± 0.00	9.00	
178	3.00 ± 0.00 3.00 ± 0.75	3.00 ± 0.00 3.00 ± 0.00	4.50 ± 0.58 4.50 ± 0.58	7.00 ± 0.00	3.50 ± 0.00	5.00 ± 0.00 5.00 ± 0.00	9.00	
	3.62	4.65	5.69	7.72	4.90	6.24		
Avg.	0.00 - 7.00	4.65 0.50–8.00	1.00-9.00	2.50-9.00	0.00-8.50	1.00-9.00	8.96 7.50–9.00	

Table 5. a. Leaf rolling index of varieties in the second screening

KCR		Early afternoon	n observation		Mor	ning observation	
code	12 DAD	14 DAD	15 DAD	16 DAD	15 DAD	17 DAD	19 DAD
114	0.00 ± 0.00	5.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00	1.50 ± 0.50	5.50 ± 0.50	6.00 ± 0.58
115	4.00 ± 0.58	7.00 ± 1.09	8.50 ± 0.87	9.00 ± 0.00	2.00 ± 0.58	7.00 ± 0.00	8.00 ± 0.58
117	4.50 ± 0.50	7.50 ± 0.64	9.00 ± 1.06	9.00 ± 0.00	5.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
118	0.75 ± 0.75	5.00 ± 1.39	8.00 ± 1.23	9.00 ± 0.00	1.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00
119	0.75 ± 0.75	4.50 ± 1.39	6.50 ± 1.17	7.00 ± 0.00	1.00 ± 0.00	4.00 ± 0.58	4.00 ± 0.58
120	3.50 ± 0.50	5.50 ± 1.04	7.50 ± 0.83	9.00 ± 0.00	4.00 ± 0.58	7.00 ± 0.00	9.00 ± 0.00
121	3.50 ± 0.50	6.00 ± 0.60	7.50 ± 0.83	9.00 ± 0.00	4.00 ± 0.58	7.00 ± 0.00	7.00 ± 0.00
122	0.00 ± 0.00	5.00 ± 1.44	9.00 ± 1.67	9.00 ± 0.00	1.00 ± 0.00	8.33 ± 0.58	8.33 ± 0.58
123	4.50 ± 0.50	5.50 ± 0.14	7.00 ± 0.64	9.00 ± 0.00	3.00 ± 0.00	6.50 ± 0.50	8.00 ± 0.58
126	7.00 ± 0.00	9.00 ± 0.67	9.00 ± 0.67	9.00 ± 0.00	6.50 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
127	1.00 ± 0.71	5.00 ± 1.11	7.00 ± 1.15	9.00 ± 0.00	5.00 ± 0.50	7.00 ± 0.00	9.00 ± 0.00
130	3.50 ± 0.50	5.00 ± 0.55	6.50 ± 0.43	9.00 ± 0.00	2.50 ± 0.96	9.00 ± 0.00	9.00 ± 0.00
132	3.00 ± 0.00	6.00 ± 1.15	7.50 ± 1.11	9.00 ± 0.00	4.50 ± 0.96	9.00 ± 0.00	9.00 ± 0.00
133	2.25 ± 0.75	4.50 ± 0.22	6.50 ± 0.68	8.00 ± 0.58	4.00 ± 1.00	6.50 ± 0.50	8.50 ± 0.50
136	6.00 ± 0.58	7.00 ± 0.55	8.00 ± 0.29	9.00 ± 0.00	5.50 ± 0.50	7.50 ± 0.50	9.00 ± 0.00
138	3.00 ± 0.00	4.50 ± 0.00	6.50 ± 0.67	8.50 ± 0.50	1.00 ± 0.00	6.50 ± 0.50	7.50 ± 0.96
139	4.00 ± 0.58	5.00 ± 0.55	7.00 ± 0.29	9.00 ± 0.00	5.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00
145	5.50 ± 0.50	9.00 ± 1.04	9.00 ± 1.01	9.00 ± 0.00	6.50 ± 0.50	9.00 ± 0.00	9.00 ± 0.00
147	4.50 ± 0.50	7.50 ± 0.95	9.00 ± 1.06	9.00 ± 0.00	5.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
148	2.25 ± 0.75	7.00 ± 1.68	9.00 ± 1.37	9.00 ± 0.00	5.00 ± 0.00	9.00 ± 00	9.00 ± 0.00
149	5.50 ± 0.50	7.00 ± 0.60	9.00 ± 0.43	9.00 ± 0.00	5.50 ± 0.96	9.00 ± 00	8.50 ± 0.50
150	0.75 ± 0.75	4.00 ± 1.31	8.50 ± 1.00	9.00 ± 0.00	2.50 ± 0.96	9.00 ± 00	9.00 ± 0.00
152	2.25 ± 0.75	7.00 ± 1.68	9.00 ± 1.37	9.00 ± 0.00	4.50 ± 0.50	9.00 ± 00	9.00 ± 0.00
153	4.00 ± 0.58	7.00 ± 0.99	9.00 ± 0.87	9.00 ± 0.00	6.00 ± 0.58	9.00 ± 00	9.00 ± 0.00
157	0.00 ± 0.00	3.50 ± 0.87	6.50 ± 1.19	7.50 ± 0.96	1.00 ± 0.00	4.50 ± 0.96	5.50 ± 1.50
158	0.00 ± 0.00	5.50 ± 2.02	7.00 ± 1.72	9.00 ± 0.00	3.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00
160	0.00 ± 0.00	6.00 ± 1.44	8.50 ± 1.72	9.00 ± 0.00	2.00 ± 0.58	7.50 ± 0.50	9.00 ± 0.00
161	0.75 ± 0.75	5.00 ± 1.39	8.50 ± 1.23	9.00 ± 0.00	1.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00
162	0.00 ± 0.00	5.00 ± 0.87	6.50 ± 1.36	9.00 ± 0.00	1.00 ± 0.00	7.50 ± 0.50	8.00 ± 0.58
163	2.00 ± 0.87	6.33 ± 1.53	7.00 ± 1.67	9.00 ± 0.00	2.33 ± 1.15	7.00 ± 0.00	8.33 ± 0.58
164	1.50 ± 0.87	3.50 ± 0.72	8.00 ± 1.26	9.00 ± 0.00	1.00 ± 0.00	6.00 ± 1.00	8.00 ± 0.58
165	0.00 ± 0.00	4.33 ± 1.44	7.67 ± 1.67	9.00 ± 0.00	1.67 ± 0.58	7.67 ± 0.58	8.33 ± 0.58
166	2.25 ± 0.75	6.00 ± 0.68	6.50 ± 1.13	9.00 ± 0.00	1.00 ± 0.00	6.50 ± 0.50	9.00 ± 0.00
168	2.25 ± 0.75	5.50 ± 0.68	8.00 ± 0.79	9.00 ± 0.00	1.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00
169	0.75 ± 0.75	4.00 ± 0.89	7.50 ± 1.00	9.00 ± 0.00	1.00 ± 0.00	6.00 ± 0.58	8.50 ± 0.50
170	1.50 ± 0.87	6.50 ± 1.23	9.00 ± 1.50	9.00 ± 0.00	1.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
171	3.67 ± 0.58	6.00 ± 0.44	9.00 ± 0.79	9.00 ± 0.00	5.50 ± 0.50	8.50 ± 0.50	8.50 ± 0.50
172	0.00 ± 0.00	6.00 ± 1.44	8.50 ± 1.91	9.00 ± 0.00	2.00 ± 0.58	8.00 ± 0.58	9.00 ± 0.00
173	1.50 ± 0.87	6.00 ± 1.23	9.00 ± 1.50	9.00 ± 0.00	2.00 ± 0.58	9.00 ± 0.00	9.00 ± 0.00
174	1.50 ± 0.87	6.50 ± 1.36	9.00 ± 1.59	9.00 ± 0.00	3.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00
175	1.50 ± 0.87	6.50 ± 1.23	8.50 ± 1.50	9.00 ± 0.00	4.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
177	3.50 ± 0.50	6.50 ± 1.11	9.00 ± 1.01	9.00 ± 0.00	5.00 ± 0.00	7.50 ± 0.50	9.00 ± 0.00
179	0.75 ± 0.75	5.00 ± 0.89	8.50 ± 1.17	9.00 ± 0.00	2.50 ± 0.96	7.50 ± 0.50	8.50 ± 0.50
180	1.50 ± 0.87	6.50 ± 1.92	9.00 ± 1.59	9.00 ± 0.00	5.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00

Table 5. b. Leaf rolling index of varieties in the second screening (continued)

KCR		Early afternoon	n observation		Mor	ning observation	
code	12 DAD	14 DAD	15 DAD	16 DAD	15 DAD	17 DAD	19 DAD
182	1.00 ± 0.87	5.67 ± 0.33	9.00 ± 1.33	9.00 ± 0.00	1.67 ± 0.58	7.00 ± 0.00	9.00 ± 0.00
183	5.00 ± 1.15	7.00 ± 1.11	9.00 ± 0.58	9.00 ± 0.00	5.50 ± 0.96	9.00 ± 0.00	9.00 ± 0.00
186	3.00 ± 0.00	6.50 ± 1.15	9.00 ± 1.11	9.00 ± 0.00	3.50 ± 0.50	9.00 ± 0.00	9.00 ± 0.00
189	0.00 ± 0.00	5.00 ± 1.44	7.00 ± 1.44	9.00 ± 0.00	1.00 ± 0.00	7.50 ± 0.50	9.00 ± 0.00
192	2.25 ± 0.75	6.50 ± 0.68	9.00 ± 1.30	9.00 ± 0.00	5.50 ± 0.50	7.50 ± 0.50	9.00 ± 0.00
193	0.75 ± 0.75	7.00 ± 1.82	9.00 ± 1.80	9.00 ± 0.00	5.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
197	0.75 ± 0.75	6.50 ± 1.82	9.00 ± 1.70	9.00 ± 0.00	5.00 ± 0.82	8.50 ± 0.50	9.00 ± 0.00
198	0.75 ± 0.00	4.00 ± 1.31	8.50 ± 1.00	9.00 ± 0.00	1.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
199	0.00 ± 0.00	6.00 ± 2.02	9.00 ± 1.91	9.00 ± 0.00	1.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
200	1.50 ± 0.87	7.00 ± 1.36	9.00 ± 1.59	9.00 ± 0.00	4.00 ± 0.58	8.50 ± 0.50	9.00 ± 0.00
202	0.75 ± 0.75	5.00 ± 1.31	8.00 ± 1.23	9.00 ± 0.00	1.50 ± 0.50	5.00 ± 0.82	8.00 ± 0.58
205	0.00 ± 0.00	5.50 ± 2.02	9.00 ± 1.72	9.00 ± 0.00	1.00 ± 0.00	6.50 ± 0.50	9.00 ± 0.00
206	0.00 ± 0.00	4.33 ± 0.87	8.00 ± 1.66	9.00 ± 0.00	2.33 ± 1.15	7.00 ± 0.00	8.33 ± 0.58
208	0.00 ± 0.00	7.00 ± 2.33	9.00 ± 2.33	9.00 ± 0.00	3.00 ± 1.73	7.00 ± 0.00	9.00 ± 0.00
209	0.00 ± 0.00	5.00 ± 1.44	8.50 ± 1.44	9.00 ± 0.00	1.00 ± 0.00	4.50 ± 0.50	8.00 ± 0.58
211	1.50 ± 0.87	3.00 ± 0.83	7.50 ± 0.43	9.00 ± 0.00	1.00 ± 0.00	4.00 ± 0.58	8.00 ± 0.58
213	3.00 ± 0.00	7.00 ± 1.33	9.00 ± 1.33	9.00 ± 0.00	4.50 ± 0.50	9.00 ± 0.00	9.00 ± 0.00
214	0.00 ± 0.00	4.00 ± 0.87	6.50 ± 1.36	7.00 ± 0.82	3.00 ± 0.00	5.50 ± 0.50	8.00 ± 0.58
216	2.25 ± 0.75	6.00 ± 1.68	8.50 ± 1.30	9.00 ± 0.00	4.50 ± 0.50	8.00 ± 0.58	9.00 ± 0.00
217	3.00 ± 0.00	5.00 ± 0.58	9.00 ± 0.58	9.00 ± 0.00	1.50 ± 0.50	6.00 ± 0.58	9.00 ± 0.00
218	0.00 ± 0.00	4.00 ± 1.44	5.00 ± 1.36	6.50 ± 0.50	1.50 ± 0.50	7.00 ± 0.00	9.00 ± 0.00
219	1.50 ± 0.87	2.00 ± 0.83	6.00 ± 0.55	9.00 ± 0.00	1.00 ± 0.00	3.50 ± 0.50	8.00 ± 0.58
220	2.25 ± 1.50	5.50 ± 1.00	7.00 ± 0.00	8.50 ± 0.50	1.50 ± 0.50	8.50 ± 0.50	9.00 ± 0.00
221	2.00 ± 1.50	6.00 ± 1.15	8.50 ± 1.00	9.00 ± 0.00	2.00 ± 0.58	5.50 ± 0.96	8.00 ± 0.58
222	3.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	4.50 ± 0.96	7.00 ± 0.00	9.00 ± 0.00
223	2.25 ± 1.50	7.00 ± 0.00	9.00 ± 0.00	9.00 ± 0.00	2.50 ± 0.96	7.00 ± 0.82	8.50 ± 0.50
225	2.00 ± 1.73	6.33 ± 1.15	9.00 ± 0.00	9.00 ± 0.00	4.33 ± 0.58	7.00 ± 0.00	9.00 ± 0.00
228	3.00 ± 0.00	6.00 ± 1.41	8.33 ± 1.15	9.00 ± 0.00	4.33 ± 0.58	8.33 ± 0.58	9.00 ± 0.00
229	1.50 ± 1.73	6.50 ± 1.00	9.00 ± 0.00	9.00 ± 0.00	4.00 ± 0.58	9.00 ± 0.00	9.00 ± 0.00
230	0.00 ± 0.00	4.00 ± 1.15	6.50 ± 1.91	8.00 ± 0.58	2.50 ± 0.96	5.00 ± 1.15	8.00 ± 1.00
234	3.00 ± 0.00	3.00 ± 0.00	5.00 ± 0.00	6.50 ± 0.50	1.50 ± 0.50	3.00 ± 0.00	5.50 ± 0.50
236	0.00 ± 0.00	2.33 ± 1.15	5.67 ± 1.15	7.67 ± 1.15	1.00 ± 0.00	5.00 ± 0.00	6.33 ± 0.58
237	0.00 ± 0.00	4.33 ± 1.15	5.67 ± 1.15	6.33 ± 0.58	2.33 ± 0.58	5.00 ± 0.00	5.00 ± 0.00
238	0.00 ± 0.00	4.50 ± 1.00	5.50 ± 1.00	7.00 ± 0.00	1.00 ± 0.00	4.00 ± 0.58	5.50 ± 0.50
239	0.00 ± 0.00	4.50 ± 1.00	6.50 ± 1.00	6.50 ± 0.50	1.50 ± 0.50	3.50 ± 0.50	5.00 ± 0.00
240	0.00 ± 0.00	1.50 ± 1.00	3.50 ± 1.00	5.00 ± 0.00	1.00 ± 0.00	4.50 ± 0.50	5.00 ± 0.82
242	0.00 ± 0.00	4.50 ± 1.00	5.00 ± 0.00	5.00 ± 0.00	1.00 ± 0.00	3.50 ± 0.50	5.00 ± 0.00
244	0.67 ± 0.58	1.00 ± 0.00	5.67 ± 1.15	7.67 ± 0.58	1.00 ± 0.00	4.33 ± 1.15	5.67 ± 0.58
245	0.00 ± 0.00	4.50 ± 1.00	7.00 ± 1.63	8.00 ± 0.58	1.00 ± 0.00	6.50 ± 0.50	7.00 ± 0.00
246	0.00 ± 0.00	1.50 ± 1.00	5.00 ± 0.00	5.00 ± 0.00	1.00 ± 0.00	4.50 ± 0.50	5.00 ± 0.82
248	0.00 ± 0.00	5.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	1.00 ± 0.00	6.00 ± 0.71	6.00 ± 0.71
A	1.74	5.41	7.79	8.50	2.82	7.07	8.18
Avg. Range	0.00-7.00	1.00-9.00	3.50-9.00	5.50-9.00	1.00-6.50	3.00-9.00	4.00-9.00

Table 6. Shoot and root characteristics of varieties in the first screening

	SDW (mg/plant)		SL (mn	SL (mm/plant)		RDW (mg/plant)		n/plant)
	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Avg.	774	260	819	525	245	111	371	304
Range	313-1397	70–517	544-1054	355–658	83-450	40-240	281 – 470	163-401

 $SDW: Shoot\ Dry\ Weight;\ SL:\ Shoot\ Length;\ RDW:\ Root\ Dry\ Weight;\ RL:\ Root\ Length.$

Table 7. Shoot and root characteristics of varieties in the second screening

	SDW (mg/plant) Control Drought		SL (mm	SL (mm/plant)		RDW (mg/plant)		n/plant)
			Control	Drought	Control	Drought	Control	Drought
Avg.	1263	190	936	472	363	88	381	335
Range	nge 257–2147 40–357		580-1323	42 – 687	123 – 657	37–153	293-543	190-492

SDW: Shoot Dry Weight; SL: Shoot Length; RDW: Root Dry Weight; RL: Root Length.

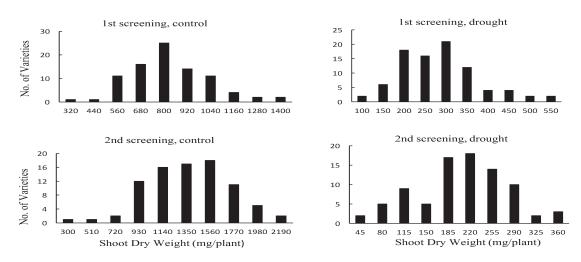


Fig. 1. Frequency distribution of shoot dry weight under control and drought conditions in the first screening and the second screening.

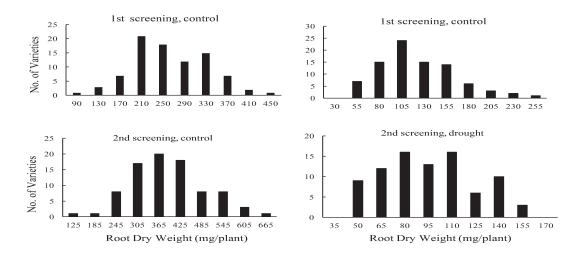


Fig. 2. Frequency distribution of root dry weight under control and drought conditions in the first screening and the second screening.

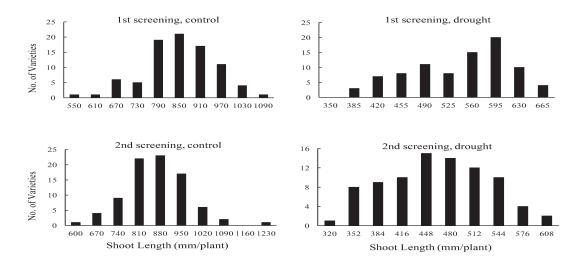


Fig. 3. Frequency distribution of shoot length under control and drought conditions in the first screening and the second screening.

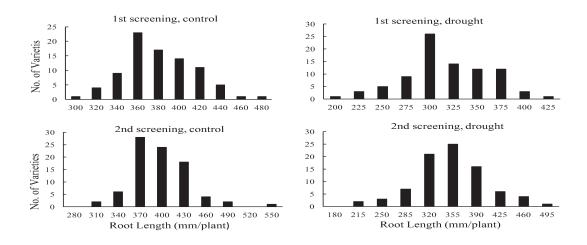


Fig. 4. Frequency distribution of root length under control and drought conditions in the first screening and the second screening.

 Table 8. Drought resistance index and drought/control ratios

 The first screen

		The first screening									
			Drought/Control								
	DRI	SDW	RDW	SL	RL						
Avg.	1.04	0.33	0.48	0.65	0.83						
Range	0.33 - 1.88	0.11 – 0.58	0.17 – 0.90	0.47 - 0.84	0.52 - 1.25						
		,	The second screening	g S							
			Drought/Control								
	DRI	SDW	RDW	SL	RL						
Avg.	1.00	0.18	0.26	0.51	0.88						
Range	0.16 – 4.22	0.03 – 0.74	0.08 – 0.79	0.05 – 0.90	0.53 - 1.34						

DRI: Drought Resistance Index; RDW: Root Dry Weight; SL: Shoot length; RL: Root length.

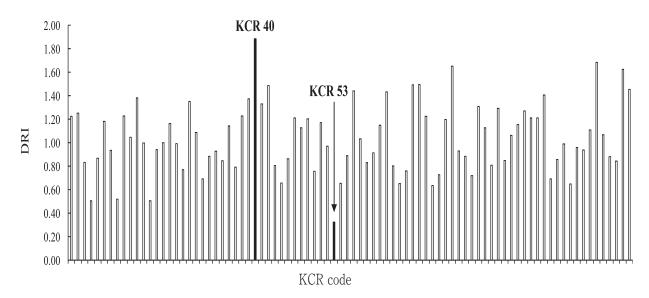


Fig. 5. Drought resistance index (DRI) of varieties in the first screening. The order of KCR code is with the smallest one in the left side as the same as Table 1.

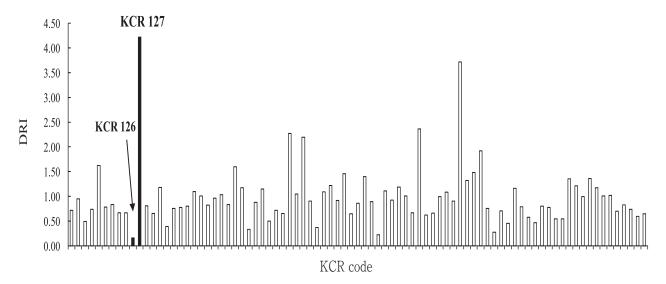


Fig. 6. Drought resistance index (DRI) of varieties in the second screening. The order of KCR code is with the smallest one in the left side as the same as Table 2.

varieties in both screening periods (Table 8, Fig. 5 and 6). DRI values recorded during the first screening ranged from 0.33–1.88 and from 0.16–4.22 during the second screening, showing greater variation during the second screening. The lowest DRI value from the first screening was 0.33 (KCR 53), while the highest value was 1.88 (KCR 40).

In the second screening, DRI was highest in KCR 127 (4.22) and lowest in KCR 126 (0.16). KCR 129 was deemed the most drought sensitive variety in the second screening, because all plants died after drought treatment.

Correlation between DRI and other parameters

DRI and drought/control ratios

We correlated DRI and drought/control ratios for

RDW (r=0.69, first screening; r=0.56, second screening), SL (r=0.47, first screening; r=0.57, second screening), and RL (r=0.20, first screening; r=0.10, second screening).

There were also positive correlations between RDW and SL (r=0.32, first screening; r=0.48, second screening), and RDW and RL (r=0.45, first screening; r=0.21, second screening), (Tables 9 and 10; Fig. 7 and 8). DRI and LRI

The relationship between DRI and LRI at different times of day is shown in Table 9 and Table 10, respectively. In general, DRI and LRI were negatively correlated except for the afternoon of 17 DAD during the second screening period. However, correlation index values were not high and varied with observation time. During the first screening period, the correlation index in the after-

Table 9. Correlation efficiencies between DRI and other parameters in the first screening

				D/C		Leaf rolling index							
		DRI	RDW	CI	DI		Early af	ternoon			Morning		
			KDW	SL	RL	12 DAT	14 DAT	15 DAT	16 DAT	15 DAT	17 DAT	19 DAT	
DRI		1.00	0.69	0.47	0.22	-0.44	-0.35	-0.34	-0.28	-0.31	-0.30	-0.01	
	RDW		1.00	0.32	0.45	-0.19	-0.18	-0.16	-0.20	-0.11	-0.13	0.01	
D/C	SL			1.00	0.14	-0.17	-0.10	-0.09	-0.18	-0.08	-0.11	-0.03	
	RL				1.00	-0.04	-0.03	-0.05	-0.12	-0.01	-0.02	-0.03	

DRI: Drought Resistance Index; RDW: Root Dry Weight; SL: Shoot Length; RL: Root Length.

D/C: Drought/Control

Table 10. Correlation efficiencies between DRI and other parameters in the second screening

				D/C		Leaf rolling index							
		DRI	RDW	CI	DI		Early af	ternoon			Morning		
			KDW	SL	RL	12 DAT	14 DAT	15 DAT	16 DAT	15 DAT	17 DAT	19 DAT	
DRI		1.00	0.56	0.57	0.10	-0.26	-0.12	-0.08	0.03	-0.04	-0.06	-0.01	
	RDW	1.00	0.48	0.21	-0.24	-0.26	-0.33	-0.23	-0.15	-0.25	-0.31	0.01	
D/C	SL		1.00	-0.17	-0.33	-0.18	-0.03	0.11	-0.15	0.00	0.03	-0.03	
	RL			1.00	0.00	0.03	-0.07	-0.10	-0.18	-0.27	-0.17	-0.03	

DRI: Drought Resistance Index; RDW: Root Dry Weight; SL: Shoot Length; RL: Root Length.

D/C: Drought/Control

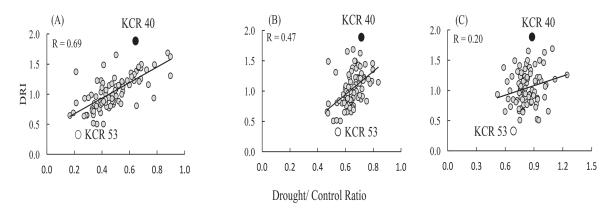


Fig. 7. Correlation between DRI and Drought/Control ratios of root dry weight (A), shoot length (B) and root length (C) in 1st screening.

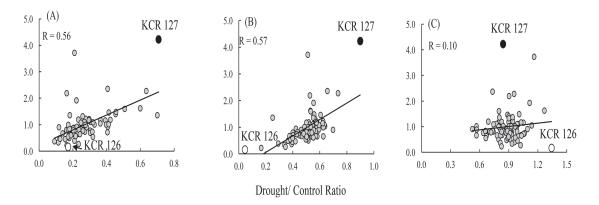


Fig. 8. Correlation between DRI and Drought/Control ratios of root dry weight (A), shoot length (B) and root length (C) in 2nd screening.

noon decreased from r=-0.44 at 12 DAD to r=-0.28 at 16 DAD. Similarly, during the second screening period, the correlation index decreased from r=-0.26 at 12 DAD to r=-0.08 at 15 DAD. The correlation index was generally higher for afternoon values than for morning values. Correlation index values for the first screening period were generally lower than for the second screening period for each day.

Negative correlations were also found between drought/control ratios for RDW, SL, RL, and LRI during both screening periods. On almost every day, the correlation between LRI and RDW was higher than that between LRI and SL and between LRI and RL.

DISCUSSION

A rapid and reliable screening method to evaluate the response of rice (*Oryza sativa* L.) to drought stress is needed for the early identification of resistant genotypes. Atlin (2003) recommended that the initial population used for evaluation should include a large number of varieties to increase the selection pool. In this experiment, we used nearly 200 varieties from various countries as a selection pool. The experiment explored the variation in drought response of different rice genotypes at the seedling stage and determined a suitable selection index for screening protocols.

Under water-limited environments, the initial stage of plant dehydration may not always be discerned. However, as the water status of the plant decreases and turgor is reduced, dehydration symptoms become recognizable. The most universally popular symptom for detecting dehydration is leaf wilting or leaf rolling. In cereals, leaf rolling is a well-recognized drought indicator. The rate of leaf rolling is being used and recommended as a reliable estimate of turgor loss in drought phenotyping of cereals (Fischer *et al.*, 2003; Bänziger *et al.*, 2000).

Blum (2011) recommended that LRI scoring should be done several times during a drought cycle to obtain an average score or a peak stress score. Scoring is usually done at noon, and maximum values are typically scored at 1:00 P.M (O'Toole, 1982). However, sometimes leaf rolling recovery can be observed the following morning.

We evaluated LRI daily, in the morning and early afternoon, throughout drought treatment. Leaf rolling was first observed in the early afternoon in both screening periods. Over the course of the drought treatment, LRI values gradually increased. Early afternoon LRI values were consistently higher than those recorded in the morning. There was a large range of LRI values between genotypes at both observation times, with a greater range in the morning than in the afternoon (Tables 4 and 5, Fig. 5 and 6).

Drought screening has been performed at both seedling and vegetative stages (De Datta, S.K. et al, 1988; Basu et al., 2010; Cui et al., 2008; Gorantla et al., 2007; Kato et al., 2008; Zhou et al., 2006). Previous results indicate that drought stress strongly affects shoot and root development in rice plants at early stages of development. Our results show that shoot and root growth

were severely depressed under drought conditions in all studied genotypes. In both the first and second screenings, RDW drought/control ratios were higher than those of SDW and the RL drought/control ratios were higher than those of SL. These results suggest that drought stress affects the shoot more severely than the root. This is in agreement with previous findings that the root/shoot ratio increases under water–limited conditions (Banba and Ookubo, 1981; Kondo et al., 2000; Kato et al., 2006).

In most cases, the target of the breeding program is increased yield, and the target at the vegetative stage is dry biomass accumulation. However, final expression of yield (or biomass) under drought stress is a complex integration of constitutive plant traits and stress-responsive processes that depend on stress intensity, duration, and timing with respect to growth stage (Blum, 2011). Therefore, in addition to absolute yield (or biomass at early stages) under stress, the percentage of yield or biomass reduction by stress is widely used to measure stress resistance in terms of plant production. We found that the average SDW drought/control ratio was 0.33 during the first screening period and 0.18 during the second screening. Drought stress on the varieties used in this experiment were very severe. Drought stress that reduces yield (or biomass) by 50% or more is considered to be severe (Lafitte, 2003). Severe stress can amplify genetic differences between varieties. Dry matter accumulation and morphology values for both screening periods and all traits showed a normal frequency distribution.

When screening a large number of varieties, individual biomass reduction for each variety is insufficient for comparison; ratios should be used instead. Fischer and Maurer (1978) proposed a DRI based on the ratio of reduction in biomass in a given genotype to the mean reduction over all genotypes in a given test. Our results showed that DRI values from the first screening (0.33–1.88) and the second screening (0.16–4.22) were highly variable, and DRI values were more variable in the second screening in the first screening.

Based on DRI values, we selected DA8 and Thierno Bande as the most drought tolerant varieties, while Malagkit Pirurutong and Pate Blanc MN1 were the most drought sensitive varieties.

Positive correlations between DRI and drought/control ratios for RDW, SL, and RL were found in both screenings. Our results show the importance of shoot and root development to drought resistance. There were also positive correlations between RDW and SL and between RDW and RL, indicating that shoot growth is correlated to root growth. However, correlation index values between DRI and drought/control ratios for RDW and SL were higher than those for RL. This suggests that, root biomass may be a more important contributor than root length to drought tolerance at the seedling stage.

There were negative correlations between DRI and LRI in both screenings, except for a single observation time. This result supports previous findings that a lack of leaf wilting is a visual symptom of drought tolerance in rice; leaf morphological evaluation is thus a useful tool for early selection. However, correlation index values

(between DRI and LRI) were not high and differed at different observation times. Breeders should therefore use LRI as a drought indicator with caution. Correlation index values were higher at early stages of drought stress than under severe drought, and higher in the afternoon than in the evening. These findings confirm previous results that leaf wilting symptoms should be observed in the early afternoon, when the stress is at a daily maximum. We further recommend leaf rolling observation during early stages of drought development.

In conclusion, there were large variations between rice varieties in LRI, shoot and root dry weight, shoot and root length, and DRI during both screening periods. Our results generally confirmed those of previous studies. In addition, we show that rapid and inexpensive screening based on leaf morphology symptoms under drought stress can be made more reliable when combined with DRI for drought resistance selection.

AUTHOR CONTRIBUTIONS

Doan Cong DIEN designed the study, gathered and analysed statistically the data, and wrote the first draft of the manuscript. Takeo YAMAKAWA designed the study, managed the analysis of parameters in the study. Takeo YAMAKAWA and Toshihiro MOCHIZUKI managed the literature search and edited the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENT

This research was supported by a Monbukagakusho Scholarship from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT). The authors thank Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University, for providing materials for this study. The authors would like to thank Agricultural Ecology Laboratory, Faculty of Agriculture, Kyushu University, for providing research facilities for this study.

REFERENCES

- Atlin G. 2003. Section 3.1. Improving drought tolerance by selecting for yield in the book "Breeding rice for drought-prone environments". Los Baños (Philippines): International Rice Research Institute
- Banba K. and Ookubo, T. 1981. Relationship between root distribution of upland crops and their yield. 3. Influence of soil moisture levels on root distribution and root dry matter of upland–cultured paddy rice, and upland rice. *Japanese Journal of Crop Science*, **50**: 1–7 (in Japanese with English abstract)
- Basu, S., A. Roychoudhury, P. P. Saha, and D. N. Sengupta. 2010. Differential antioxidative responses of Indica rice cultivars to drought stress. *Plant Growth Regulation*, 60(1): 51–59
- Blum, A., 1988. Plant Breeding for Stress Environments. Springer Blum, A., 2011. Plant breeding for water–limited environments. Springer
- Cui, K., J. Huang, Y. Xing, S. Yu, C. Xu, and S. Peng. 2008. Mapping

- QTLs for Seedling Characteristics under Different Water Supply Conditions in Rice (*Oryza sativa* L.). *Physiologia Plantarum*, **132**(1): 53–68
- De Datta, S. K., Malabuyoc, J. A. and Aragon, E. L., 1988. A field screening technique for evaluating rice germplasm for drought tolerance during the vegetative stage. Field Crops Research, 19(2): 123–134
- Fischer KS, Lafitte R, Fukai S, Atlin G, Hardy B, editors. 2003. Breeding rice for drought–prone environments. Los Baños (Philippines): International Rice Research Institute
- Fischer RA, Maurer R. 1978. Drought resistance in spring wheat cultivar. I. Grain yield responses. Australian Journal of Agricultural Research, 29(5): 897–912
- Fukai S, G Pantuwan, B Jongdee, M Cooper. 1999. Screening for drought resistance in rainfed lowland rice. Field Crops Research, 64(1-2): 61-74
- Gorantla, M., P. R. Babu, V. B. R. Lachagari, A. M. M. Reddy, R. Wusirika, J. L. Bennetzen, and A. R. Reddy. 2007. Identification of Stress-responsive Genes in an Indica Rice (Oryza sativa L.) using ESTs GRiSP (Global Rice Science Partnership). 2013. Rice almanac, 4th edition. Los Baños (Philippines): International Rice Research Institute
- Hsiao, T. C., J. C. O'Toole, E. B. Yambao and N. C. Turner, 1984.
 Influence of osmotic adjustment on leaf rolling and tissue death in rice. *Plant Physiology*, 75(2): 328–341
- Kato, Y., Abe, J., Kamoshita, A. and Yamagishi, J. 2006. Genotypic variation in root growth angle in rice (*Oryza sativa* L.) and its association with deep root development in upland fields with different water regimes. *Plant and Soil*, 287(1): 117–129
- Kato, Y., S. Hirotsu, K. Nemoto, and J. Yamagishi. 2008. Identification of QTLs Controlling Rice Drought Tolerance at Seedling Stage in Hydroponic Culture. *Euphytica*, **160**(3): 423–430
- Kondo, M., Maddala, V. R. and Aragones, D. V. 2000. Characteristics of root growth and water uptake from soil in upland ice and maize under water stress. Soil Science and Plant Nutrition, 46(3): 721–732
- Kumar A, Verulkar SB, Dixit S, Chauhan B, Bernier J, Venuprasad R, Zhao D, Shrivastava MN. 2009. Yield and yield–attributing traits of rice (*Oryza sativa* L.) under lowland drought and suitability of early vigor as a selection criterion. *Field Crops Research*. 114(1), 9–107
- Lafitte R, A. Blum, and G. Atlin. 2003. Breeding rice for drought-prone environments. Los Baños (Philippines): International Rice Research Institute pp. 43–54
- Loresto, G. C., T. T. Chang and 0. Tagumpay, 1976. Field evaluation and breeding for drought resistance. *Philippine Journal of Crop Science*, **1**(1): 36–39
- Loresto, G. C. and T. T. Chang, 1981. Decimal scoring systems for drought reaction and recovery ability in rice screening nurseries. International Rice Research Institute. Newsletter, ${\bf 6}(2)$: 9–10
- O'Toole, J. C., 1982. Drought resistance in crops with emphasis on rice. International Rice Research Institute, P. O. Box 933, Manila, Philippines. pp. 195–213
- O'Toole, J. C. and T. T. Chang, 1978. Drought and rice improvement in perspective. IRRI Research Paper Series, No. 14. International Rice Research Institute
- O'Toole, J. C. and R. T. Cruz, 1980. Response of leaf water potential, stomatal resistance, and leaf rolling to water stress. *Plant Physiology*, **65**(3): 428–432
- O'Toole, J. C., R. T. Cruz and T. N. Singh, 1979. Leaf rolling and transpiration. *Plant Science Letters*, **16**(1): 111–114
- Zhou, S. X., F. Tian, Z. F. Zhu, Y. C. Fu, X. K. Wang, and C. Q. Sun. 2006. Identification of Quantitative Trait Loci Controlling Drought Tolerance at Seedling Stage in Chinese Dongxiang Common Wild Rice (*Oryza rufipogon Griff.*). Acta Genetica Sinica, 33(6): 551–558