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Evaluation of Cold Tolerance in NERICAs Compared with Japanese Standard Rice Varieties at the Reproductive Stage

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Abstract

New Rice for Africa (NERICA) is a general name for interspecific rice varieties derived from a cross between the high-yielding Asian rice (*Oryza sativa* L.) between locally adapted African rice (*Oryza glaberrima* Steud.). Eight NERICAs were evaluated for cold tolerance (CT) at the reproductive stage and compared with their *O. sativa* parents and three Japanese standard rice varieties over 3 years. Cold tolerance was evaluated based on the filled grain ratio (FGR) after cold water irrigation. The FGR was greatly reduced by cold water irrigation. NERICA 1, 2 and 7 had higher FGR (51.9–57.9 %), while NERICA 6, 15 and 16 had lower FGR (6.2–14.5 %). NERICA 1, 2 and 7 were less affected by cold stress, with a 31 % mean reduction in FGR, while NERICA 6, 15 and 16 were greatly affected, with their FGRs being reduced by more than 80 %. NERICA 3 and 4 were moderately affected by cold stress, with about 45 % reduction rate in FGR. FGR significantly influenced the grain weights of the varieties with strong positive correlations ($r = 0.83$–0.91; $P < 0.001$), and thus, similar trends in grain weights were observed. Grain weights were reduced by 61.7–96.4 % under cold stress. NERICA 1, 2 and 7 showed significantly better performance than NERICA 3 and 4, while NERICA 6, 15 and 16 performed poorly under cold water irrigation. The Japanese varieties Koshihikari (very tolerant) and Ozora (moderately tolerant) were more affected by cold water irrigation than NERICA 1, 2 and 7. On the basis of the mean reduction rate (%) in FGR under cold stress, the varieties were classified as follows: NERICA 1, 2 and 7 as tolerant; NERICA 3 and 4 as moderately tolerant; and NERICA 6, 15 and 16 as susceptible to cold stress. However, NERICA 7 grain yields were lower under cold stress due to both greatly reduced number of panicles per plant and number of spikelets per panicle. Therefore, NERICA 1 and 2 are suitable candidates for production in the highland regions of East Africa and should be promoted for production.

Introduction

New Rice for Africa (NERICA) is a general name for interspecific rice varieties derived from a cross between the high-yielding Asian rice (*Oryza sativa* L.) with locally adapted African rice (*Oryza glaberrima* Steud.). NERICA varieties were developed at the Africa Rice Center (WARDA) as interspecific BC_2 inbred lines that combine the best traits from various cross combinations between the two species of cultivated rice (Jones et al. 1997). Seven

NERICA varieties (NERICA 1–7) were released in 2000, and an additional 11 varieties (NERICA 8–18) were released in 2005; all 18 varieties are suitable for the upland rice ecology of sub-Saharan Africa (Africa Rice Center/ FAO/SAA 2008). The promotion of NERICA rice production, particularly under rain-fed conditions in high-altitude regions in East Africa, has been given considerable attention to boost rice production. Generally, highlands in East Africa are agriculturally rich regions occurring at high altitudes (over 900 m above sea level) and are characterized by cooler temperatures and plentiful precipitation, thus, having huge potential for rice production. Because of their elevation, low-temperature damage could occur, resulting in considerable yield losses. The cultivation of rice at high-elevation regions in Africa as a whole, therefore, will be threatened by cold stress at various growth stages.

On the other hand, especially in high-altitude regions in East Africa, temperature gradually decreases during the latter half of long rainy season and reproductive stage during rice growth coincides with these low-temperature conditions in these regions. Low-temperature stress is an important factor affecting the growth and development of rice in temperate and high-elevation areas in tropical environments (Shimono et al. 2002, Andaya and Mackill 2003). Low temperatures, especially during panicle development and the booting stage, decrease the spikelet fertility of rice (Shimono et al. 2002, Gunawardena et al. 2003b), resulting in grain yield losses. Spikelet sterility is a symptom of adverse growing conditions on reproductive development, which reduces grain yields in rice (Gunawardena et al. 2003a). Yield losses in rice due to low temperatures at high-latitude and high-altitude areas have been well documented in many countries such as Japan (Shimono et al. 2007), Korea (Lee 2001), China (Dai et al. 2004, Xu et al. 2008, Jiang et al. 2010) and Australia (Farell et al. 2001).

At the reproductive stage, cold causes abnormal spikelet development and reduces their fertility (Jacobs and Pearson 1999). Poor panicle exertion, inhibited anther dehiscence and pollination, and even spikelet abortion may occur (Lee 2001). At maturity, early leaf senescence and poor grain filling and quality occurs (Gunawardena et al. 2003a). Cold stress reduces photosynthesis, resulting in low dry matter accumulation (reduced growth), indirectly suppressing rice yields. The carbohydrates available for grain production become limited, leading to low yields (Jacobs and Pearson 1999). The reproductive phases of growth, which include the panicle initiation, booting and flowering stages, are the most critical for determining final grain yield. The booting stage is the most sensitive to cold injury in rice, especially the early pollen microspore stage, which occurs approximately 10–12 days before heading (Satake 1976). Low temperatures at the booting stage cause anther injury, impair pollen development and increase the number of aborted microspores, resulting in high spikelet sterility, and therefore, decreased rice yields (Satake 1989). Cold tolerance at the reproductive phase of growth, which includes the booting and flowering stages, has been evaluated on the basis of the percentage of fertile spikelets after cold water irrigation (Saito et al. 1995, Takeuchi et al. 2001). Rice grain yields are severely reduced by low water temperatures during the reproductive period as a result of low spikelet fertility (Shimono et al. 2002).

Rice cultivars vary greatly in their cold tolerance. In tropical environments, the *indica* rice subspecies is the most common, and it is more sensitive to low temperatures (Andaya and Mackill 2003) than the *japonica* subspecies, which is divided into tropical and temperate groups (Glazsmann et al. 1990, Mackill and Lei 1997, Nakagahra et al. 1997, Garris et al. 2005). On the other hand, despite the intensified efforts for rain-fed NERICA production in the East African highlands, their cold tolerance ability has not been evaluated, and information on their suitability for production in high altitudes regions is scarce. The objective of this study was to evaluate the cold tolerance of eight NERICA varieties and their recurrent parents (*O. sativa*) at the reproductive stage in comparison with Japanese standard rice varieties in a paddy field irrigated with water from a cold stream in a mountainous region in Japan. The study provides information on the suitability of NERICA production in highland regions, including the varieties that are the most promising. This information also has practical benefits for rice breeding programmes, such as the identification of new sources of germplasm and plant breeding materials for improving cold tolerance in rice.

Materials and Methods

Plant materials

Eight NERICA varieties (Africa Rice Center/FAO/SAA 2008) and three Japanese standard rice varieties that were produced by cross-breeding using different Japanese traditional varieties (Koshihikari, very tolerant; Ozora, moderately tolerant; Tsukinohikari, susceptible; heading in mid to late August) used for cold tolerance evaluation at the Aichi Prefecture Mountainous Research Institute were used in this study (Table 1). Two *Oryza sativa* parents of NERICAs, WAB56-104 and WAB181-18, were also planted in 2010 and 2011 (Table 1). WAB56-104 is the parent of NERICA 1–11, and WAB181-18 is the parent of NERICA 15–18 (Jones et al. 1997).

Field evaluation of cold tolerance

Field experiments were conducted in a paddy field irrigated with water from a cold stream (Fig. 1) at the Aichi

Table 1 Mean water temperatures in the cold water irrigation paddy field in 2009–2011

	Temperature (°C)		
	2009	2010	2011
Maximum	20.3	21.3	21.1
Average	19.0	20.0	19.6
Minimum	17.9	18.9	18.4

Fig. 1 Paddy field irrigated with water from a cold stream. Arrows indicate the direction of flow of cold water.

Prefecture Mountainous Research Institute, Japan (latitude: 35°13′N, longitude: 137°E, 505 m a.s.l) in 2009, 2010 and 2011. Germinated seeds were sown in seedling trays in early May each year and transplanted at the 4–5 leaf stage (on 6 June in 2009, 2010 and 2011) in single rows at a spacing of 30 cm between rows and 15 cm between plants, one plant per hill and 10 hills per variety. Control paddy fields irrigated with normal water conditions (21–29 °C) were also planted. The field plantings under both cold and normal water irrigation followed a randomized complete block design with two replicates. Details of the cold water treatment period and temperature are presented in Fig. 2 and Table 1.

Measurements

Sampling was done starting with the third plant in each row. Five hills of each variety were sampled from each of the blocks under cold and normal water conditions and were used for the following measurements. Plant height was measured from the soil to the tip of the flag leaf at maturity. Heading date was measured as the date of 50 % heading. The number of panicles per plant was counted. Straw dry weight was determined after air-drying using a weighing balance (EK610i; A and D Co. Ltd., Tokyo, Japan). The panicles were hand-threshed, and filled spikelets were separated from unfilled ones by floatation in water. They were then dried in the sun for a few hours and weighed separately. The moisture content of the filled spikelets was measured using a grain moisture tester (Riceter f; Kett Electric Laboratory, Tokyo, Japan). Grain weight per plant adjusted to 14 % moisture content was calculated from weighed filled spikelets in each hill and the measured moisture values. The total number of filled and unfilled spikelets was counted. The per cent filled grain ratio (FGR) was calculated as 100 times the number of filled spikelets divided by the total number of spikelets. The number of spikelets per panicle was also calculated. Cold tolerance was evaluated mainly on the basis of the percentage of filled grains, and classification was done based on the per cent reduction rate in FGR (Cruz et al. 2006, Ye et al. 2009, Suh et al. 2010).

Statistical analysis

An analysis of variance (ANOVA) was conducted on the data for each trait to compare the performance of NERICA varieties with those of their parents and Japanese standard varieties. ANOVA was performed using the GLM procedure, and correlation analysis between the agronomic traits was performed using the correlation procedure in SAS program (SAS version 9.1; SAS Institute, Cary, NC, USA). Data for the mean FGR and per cent reduction rates were arcsine-transformed, and means are reported after back-transforming. Means were separated by least significant difference test at $P < 0.05$.

Results

Water temperature in the cold irrigation paddy field

The water temperature during the treatment period in 2009 (July 2–September 1) had ranges of 18.4–25.8, 17.3–20.5 and 16–19.4 °C for the maximum, average and minimum temperatures, respectively (Fig. 2). The mean temperatures in 2009 were 20.3, 19 and 17.9 °C for the maximum, average and minimum, respectively (Table 1). The water temperature during the treatment period in 2010 (July 1–August 30) had ranges of 17.7–26.7, 16.6–20.8 and 16.5–19.7 °C for the maximum, average and minimum temperatures, respectively (Fig. 2). The mean temperatures in 2010 were 21.3, 20 and 18.9 °C for the maximum, average and minimum, respectively (Table 1). The water temperature during the treatment period in 2011 (July 12–September 2) had ranges of 17.5–25.9, 17.2–22.5 and 16.5–20.1 °C for the maximum, average and minimum temperatures, respectively (Fig. 2). The mean temperatures in 2011 were 21.1, 19.6 and 18.4 °C for the maximum, average and

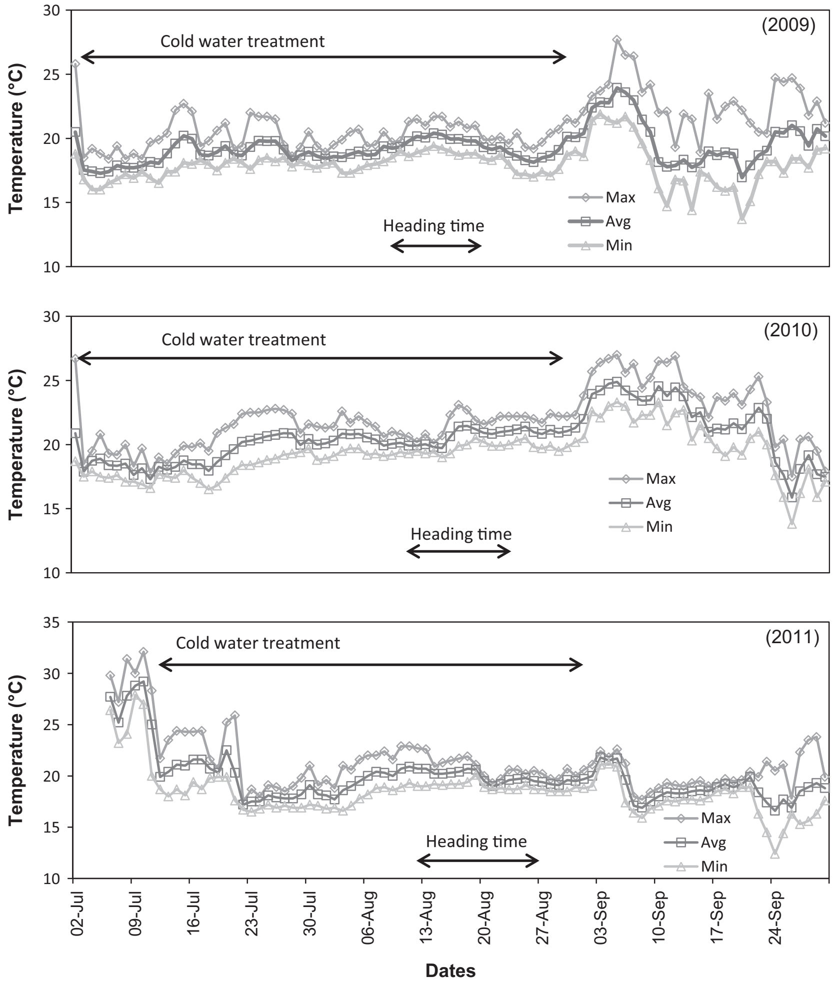

Fig. 2 Water temperature in the cold water irrigation paddy field in 2009, 2010 and 2011. Max, Avg, and Min represent the maximum, average and minimum temperatures, respectively.

minimum, respectively (Table 1). Water temperatures were lower in 2009 and 2011 than in 2010. The mean average temperature was lower by 1 °C in 2009 and by 0.4 °C in 2011 compared with the mean average temperature in 2010 (Table 1).

Agronomic and reproductive characteristics

For all the traits measured, interaction effect between years and varieties were significant under cold stress (Table S1). Performance of the varieties in terms of these traits was thus analysed and presented for each year separately.

Heading dates

Heading dates for the NERICAs and other varieties were between August 10 and 19 in 2009, August 12 and 23 in 2010, and August 12 and 27 in 2011 (Table 3), under cold water irrigation. Cold water irrigation started 30 days before heading time for all varieties in all years (Fig. 2), and thus, the treatment periods were within the critical stage for cold injury to occur (Satake 1976). Therefore, the timing of cold water irrigation in this study was effective for cold tolerance evaluation at the reproductive stage.

Plant height

The NERICAs and their parents were significantly taller than the Japanese varieties. Under normal water conditions, NERICAs measured 105.8–129.6 cm in height and Japanese varieties measured 95.8–108 cm in height ($P < 0.001$; Table 2). In the cold-irrigated paddy field, NERICAs had significantly taller plants with heights ranging from 93 to 119.7 cm compared with the Japanese varieties' height range of 81.8–91.6 cm at harvest ($P < 0.001$; Table 4). Plant height was reduced by cold water irrigation by 1.2–16.1 %, with the exception of NERICA 6, whose height was not affected by cold stress (Table 5). Plant height in most of the NERICA varieties and their parents, except NERICA 16, was less affected by cold water irrigation compared with the Japanese varieties (Table 5).

Table 2 Reproductive and agronomic characteristics of NERICA and Japanese standard rice varieties under normal water irrigation

Variety	Plant height at harvest (cm)	Straw dry weight at harvest (g)	No. of panicles per plant	Grain weight (g/plant)	No. of spikelets per panicle	Filled grain ratio (%)	Cold tolerance
Koshihikari	108.0 de	32.3 a	12 a	24.0 abc	102 ef	89.5 a	VT
Ozora	97.4 f	26.8 abc	13 a	29.0 a	99 f	92.0 a	MT
Tsukinohikari	95.8 f	30.2 ab	12 a	23.4 abcd	90 f	86.7 ab	S
WAB56-104	118.9 bc	26.8 abc	6 bcd	26.9 ab	177 a	89.5 a	–
NERICA 1	107.0 e	22.2 c	6 bcd	17.4 cdef	126 de	86.5 ab	–
NERICA 7	121.8 b	25.2 abc	7 bc	19.9 bcdef	131 cd	78.8 cd	–
NERICA 2	105.8 e	24.1 bc	7 bc	16.5 def	142 bcd	77.5 d	–
NERICA 4	115.6 bc	25.2 bc	8 b	20.9 bcde	148 bcd	76.8 d	–
NERICA 3	114.2 cd	24.7 bc	7 bc	22.3 abcde	154 abc	80.5 cd	–
NERICA 6	115.2 bc	25.6 abc	5 cd	13.3 f	158 ab	66.0 e	–
WAB181-18	115.2 bc	23.2 bc	6 bcd	26.4 ab	175 a	83.4 bc	–
NERICA 15	119.8 bc	21.1 c	4 d	15.5 ef	149 bcd	83.3 bc	–
NERICA 16	129.6 a	25.3 abc	5 cd	22.0 abcde	159 ab	87.7 ab	–

Means followed by the same letter within a column are not significantly different (GLM procedure in SAS, $P < 0.05$). VT, very tolerant; MT, moderately tolerant; S, susceptible; –,unknown; NERICA, New Rice for Africa.

Panicle number

The number of panicles per plant was consistent in the 3 years, with NERICAs and their parents having significantly fewer panicles than the Japanese varieties. Under normal water conditions, NERICAs and their parents had 4–8 panicles per plant, as compared to the 12–13 panicles per plant in Japanese varieties ($P < 0.001$; Table 2). A similar trend was observed under cold water irrigation with NERICAs and their parents having 3–5 panicles per plant, which were significantly lower than the range of 7–12 for the Japanese varieties ($P < 0.001$; Table 4). Cold water irrigation reduced the number of panicles by 1–4 in NERICAs and by 3–6 in the Japanese varieties. The per cent reduction in the number of panicles was higher in NERICA 3, 4 and 7 and Tsukinohikari and lower in Koshihikari (Table 5).

Number of spikelets per panicle

The NERICAs and their parents had significantly more spikelets per panicle compared with the Japanese standard varieties. Under normal water conditions, NERICAs and their parents had 126–177 spikelets per panicle, while Japanese varieties had 90–102 spikelets per panicle ($P < 0.001$; Table 2). NERICAs and their parents had 79–165 spikelets per panicle, while Japanese varieties had 82–107 spikelets per panicle under cold water irrigation ($P < 0.001$; Table 4). Cold water irrigation reduced the number of spikelets per panicle by 4.5–30.8 %. The number of spikelets per panicle was least affected by cold stress in NERICA 2, 3 and 4 and Tsukinohikari, with a reduction of 0–5.3 %, and most affected in NERICA 6, 7, 15 and 16 and Koshihikari with a reduction of 26.2–30.8 % (Table 5). Generally, the varieties with the greatest reduction in the number of panicles after cold water irrigation had smaller reductions in the number of spikelets per panicle except for NERICA 7.

Straw dry weight

Under normal water conditions, Japanese varieties and NERICA parents (except WAB181-18) had higher straw dry weight, ranging from 26.8 g/plant to 32.3 g/plant, than NERICAs, which had a range of 21.1 g/plant to 25.6 g/plant (Table 2). Under cold irrigation, dry straws of NERICAs and their parents weighed 11.9–22.1 g/plant and those of Japanese varieties weighed 14.5–19.9 g/plant across the 3 years (Table 3). Straw dry weight was significantly higher in NERICA 6 and Tsukinohikari and the lowest in NERICA 15 (Table 4). Cold water irrigation reduced straw dry weight by 25.2–52.6 %. Straw dry weight was least affected by cold stress in NERICA 6, with a reduction of 25.2 %, and most affected in Koshihikari and WAB56-104, with a reduction of 48.9–52.6 % (Table 5).

Filled grain ratio

Under normal water conditions, the FGR ranged from 66–92 %, with the Japanese varieties and NERICA parents having higher FGRs than the NERICAs (Table 2). Under cold water irrigation, FGR varied significantly among the varieties, from as low as 0.6 % up to 78.6 % across the 3 years ($P < 0.001$; Table 3). FGR was more severely affected in 2009 and 2011 than in 2010, and this was attributed to the mean temperature differences during the treatment period in the 3 years; the mean temperature was lower by 1 °C in 2009 and by 0.4 °C in 2011 than in 2010 (Table 1). Among the NERICAs, NERICA 1, 2 and 7 showed higher FGRs, while NERICA 6, 15 and 16 showed lower FGRs (Table 3). WAB56-104 had a significantly higher FGR

Table 3 Reproductive characteristics of NERICA and Japanese standard rice varieties under cold water irrigation in 2009-2011

		Filled grain ratio (%)				Grain weight (g/plant)				Heading date		
Variety	Cold tolerance	2009	2010	2011	Mean	2009	2010	2011	Mean	2009	2010	2011
Koshihikari	VT	28.9 b	64.8 bc	7.0 e	35.9	4.2 a	8.6 a	1.1 fg	4.9	16-Aug	13-Aug	14-Aug
Ozora	MT	8.6 de	35.5 ef	12.9 d	24.2	1.1 c	3.9 cd	2.0 ef	3.0	16-Aug	18-Aug	17-Aug
Tsukinohikari	S	0.9 f	10.9 hi	4.9 ef	7.9	0.1 c	1.0 e	0.7 fg	0.9	12-Aug	20-Aug	23-Aug
WAB56-104	–		78.6 a	65.4 a	72.0		10.1a	10.5 a	10.3		17-Aug	19-Aug
NERICA 1	–	37.6 a	68.8 ab	47.0 b	57.9	3.6 ab	5.7 bc	4.7 cd	5.2	12-Aug	19-Aug	27-Aug
NERICA 7	–	35.0 ab	61.6 bc	44.2 bc	52.9	4.6 a	6.1 b	3.5 de	4.8	10-Aug	15-Aug	16-Aug
NERICA 2	–	33.8 ab	56.5 cd	47.4 b	51.9	3.5 ab	5.7 bc	5.9 bc	5.8	16-Aug	23-Aug	27-Aug
NERICA 4	–	30.1 b	42.4 e	47.6 b	45.0	3.5 ab	4.7 bc	7.5 b	6.1	12-Aug	14-Aug	16-Aug
NERICA 3	–	19.1 c	45.3 de	39.7 c	42.5	2.6 b	5.6 bc	6.3 bc	5.9	13-Aug	16-Aug	12-Aug
NERICA 6	–	1.3 ef	7.7 i	4.7 ef	6.2	0.1 c	0.5 e	0.4 fg	0.5	19-Aug	19-Aug	27-Aug
WAB181-18	–		20.3 gh	5.6 ef	12.9		2.2 de	0.8 fg	1.5		17-Aug	12-Aug
NERICA 15	–	0.6 f	27.3 fg	1.7 f	14.5	0.1 c	1.8 de	0.1 g	1.0	10-Aug	16-Aug	16-Aug
NERICA 16	–	10.1 d	19.6 gh	6.4 ef	13.0	1.1 c	1.8 de	0.6 fg	1.2	10-Aug	12-Aug	16-Aug

Means followed by the same letter within a column are not significantly different (GLM procedure in SAS, $P < 0.05$). Mean indicates average values for 2010 and 2011 data. VT, very tolerant; MT, moderately tolerant; S, susceptible; –,unknown; NERICA, New Rice for Africa.

Table 4 Agronomic characteristics of NERICA and Japanese standard rice varieties under cold water irrigation in 2009-2011

	Plant height at harvest (cm)			Straw dry weight at harvest (g)			No. of panicles per plant			No. of spikelets per panicle		
Variety	2009	2010	2011	2009	2010	2011	2009	2010	2011	2009	2010	2011
Koshihikari	91.6 c			17.7 bcd	14.5 cde	16.1 bc	8 a	7 a	12 a	82 de	84 de	66 e
Ozora	82.9 d			17.6 bcd	17.7 abc	14.4 bc	8 a	8 a	8 b	87 de	78 e	85 de
Tsukinohikari	81.8 d			18.4 abc	19.9 a	15.9 bc	9 a	7 a	7 b	82 de	87 de	107 bcd
WAB56-104		108.0 bc	111.0 bc		12.9 de	14.5 bc		4 c	4 c		130 ab	165 a
NERICA 1	93.0 c	101.5 d	102.3 e	17.5 bcd	12.1 e	15.4 bc	4 bcd	3 c	4 c	82 de	89 de	121 b
NERICA 7	109.3 a	110.1 b	105.7 de	21.3 ab	15.4 bcde	13.4 c	5 bc	4 c	4 c	82 de	90 de	94 cd
NERICA 2	100.0 b	101.1 d	106.2 de	16.6 cd	12.6 de	16.5 b	4 bcde	5 bc	5 c	123 a	111 bc	159 a
NERICA 4	98.6 b	110.6 b	117.7 a	16.8 cd	12.2 e	16.4 b	5 bc	4 c	4 c	95 cd	132 a	165 a
NERICA 3	102.2 b	103.5 cd	119.7 a	19.2 abc	13.9 cde	15.3 bc	5 b	4 c	4 c	113 ab	141 a	153 a
NERICA 6	109.8 a	117.3 a	119.5 a	22.1 a	18.5 ab	19.7 a	4 cde	3 c	4 c	104 bc	94 cde	125 b
WAB181-18		100.6 d	109.1 bcd		15.2 bcde	13.5 c		4 c	5 c		143 a	154 a
NERICA 15	100.9 b	112.1 b	112.9 b	12.3 e	11.9 e	14.0 bc	3 de	3 c	4 c	79 e	95 cde	122 b
NERICA 16	102.2 b	109.6 b	107.8 cd	13.9 de	16.4 abcd	15.2 bc	3 e	4 c	3 c	90 de	100 cd	120 bc

Means followed by the same letter within a column are not significantly different (GLM procedure in SAS, $P < 0.05$). NERICA, New Rice for Africa

than the NERICAs (65.4–78.6 %), while WAB181-18 had significantly lower FGR of 5.6–20.3 %.

Cold water irrigation greatly reduced the FGR in all the varieties, with greater reductions in 2009 and 2011 than in 2010 (Table 7). The differences in the per cent reduction in FGR were consistent in the 3 years (Table 7). Since the cold treatment was very severe in 2009 (Table 1), we considered the mean percentage reduction for 2010 and 2011 (Table 7). Significantly greater reductions by more than 80 % were observed in NERICA 6, 15 and 16; WAB181-18; and Tsukinohikari. FGR was reduced by 59.9–73.7 % in Koshihikari and Ozora and by 41.3–47.4 % in NERICA 3 and 4. FGR was reduced by 30.7–31.7 % in NERICA 1, 2 and 7. WAB56-104 was superior to the NERICAs, with a reduction of <20 %. The results indicate that NERICA 1, 2 and 7 performed better under cold water than NERICA 3 and 4, while NERICA 6, 15 and 16 performed very poorly. WAB56-104 was more tolerant than the NERICAs, while WAB181-18 was very susceptible to cold stress. The genomic contribution of the two parents to their progenies might explain the trend in the performance of NERICAs under cold water irrigation. However, NERICA 6 showed a different response to cold water compared with the other progenies of a similar parent.

Grain weight per plant

Similar trends to those for FGR were observed for grain weight per plant. Under normal water conditions, the

Table 5 Percent reduction rate (%) of measured agronomic characteristics of NERICA and Japanese standard rice varieties after cold water irrigation. Data are mean values for 2010 and 2011

Variety	Plant height	Straw dry weight	No. of panicles per plant	No. of spikelets per panicle	Grain weight
Koshihikari	15.2 a	52.6 a	17.8 g	26.2 abc	79.8 c
Ozora	14.9 a	40.2 bc	38.3 bcde	17.6 bcd	89.8 b
Tsukinohikari	14.6 a	40.8 bc	42.9 abc	−7.7 g	96.3 a
WAB56-104	7.9 cd	48.9 ab	33.1 cdef	17.0 bcde	61.7 g
NERICA 1	4.8 ef	38.0 c	37.5 bcde	16.5 bcde	70.1 ef
NERICA 7	11.4 b	42.9 bc	44.9 ab	29.5 ab	76.0 cd
NERICA 2	2.0 gf	39.5 c	31.8 def	5.3 de	64.8 fg
NERICA 4	1.2 g	43.1 bc	50.6 a	−0.3 fg	70.9 de
NERICA 3	2.3 gf	41.0 bc	41.4 abcd	4.5 efg	73.5 de
NERICA 6	−2.8 h	25.2 d	30.9 def	30.8 a	96.4 a
WAB181-18	9.0 bc	38.1 c	29.0 ef	15.3 cde	94.1 ab
NERICA 15	6.1 de	38.7 c	26.1 fg	27.0 abc	93.7 ab
NERICA 16	16.1 a	37.5 c	35.2 bcdef	30.8 a	94.5 ab

Means followed by the same letter within a column are not significantly different (GLM procedure in SAS, $P < 0.05$). NERICA, New Rice for Africa

Japanese varieties and NERICA parents had higher grain weights than NERICAs, ranging from 23.4 g/plant to 29 g/plant, compared with 13.3 g/plant to 22.3 g/plant in NERICAs (Table 2). Under cold water irrigation, grain weight per plant varied significantly among the varieties from 0.1 to 10.5 g/plant across the 3 years ($P < 0.001$; Table 3). NERICAs had grain weights of 0.1–7.5 g/plant, and Japanese standard varieties had grain weights of 0.1–8.6 g/plant. WAB56-104 had a significantly higher grain weight than the NERICAs of 10.1–10.5 g/plant, while WAB181-18 had a significantly lower grain weight of 0.8–2.2 g/plant. Grain weight was influenced by the percentage of filled grains, with varieties having higher percentage of filled grains showing higher grain weight than those with lower percentages of filled grains ($r = 0.83$–0.91; $P < 0.001$).

Cold water irrigation greatly reduced grain weight in all the varieties. Grain weights were reduced by more than 60 % in all the varieties, with the greatest reductions (93.7–96.4 %) in NERICAs 6, 15 16, WAB181-18 and Tsukinohikari. WAB56-104 had the lowest reduction of 61.7 %, while WAB181-18 was greatly affected, with its grain weight being reduced by 94.1 % (Table 5). The results indicate that under cold stress, there was great yield reductions in all the varieties used in this study. The duration of the cold effect was prolonged during the grain-filling stage (Fig. 2), which may have affected the grain filling, resulting in the high reduction in grain weights under cold water irrigation. WAB56-104 had better yields than the NERICAs, while WAB181-18 performed poorly, and their progenies showed similar results with the exception of NERICA 6.

Relationship between FGR, grain weight and other agronomic traits

A high positive correlation was observed between the FGR and grain weight per plant in all the years (Table 6). High FGR significantly resulted to increased grain weights, indicating that the FGR is a major factor determining rice grain yields. Therefore, to increase grain yields in cold-prone environments, more attention should be directed to improving the FGR in rice cultivars at the reproductive stage. On the other hand, significant positive correlations were also observed between grain weight and number of panicles per plant in 2010 and number of spikelet per panicle in 2011 (Table 6). This means that these traits are also

Table 6 Correlation coefficients (r) between the filled grain ratio and other agronomic traits under cold water irrigation

	Filled grain ratio (%)			Grain weight		
	2009	2010	2011	2009	2010	2011
Grain weight per plant	0.89 ***	0.83 ***	0.91 ***			
Straw dry weight	0.01 ns	−0.45 **	−0.09 ns	0.03 ns	−0.12 ns	0.06 ns
No. of panicles per plant	0.04 ns	0.07 ns	−0.24 ns	0.24 ns	0.37 **	−0.11 ns
No. of spikelets per panicle	0.12 ns	−0.16 ns	0.19 **	0.12 ns	0.06 ns	0.42 ***

**$P < 0.01$, ***$P < 0.001$. ns, not significant.

important to maintain grain yields under cold-prone environments.

Classification of cold tolerance

Due to the significant effect of FGR on grain weights of the varieties (r = 0.83–0.91; $P < 0.001$), trends in FGR reduction and grain weight reduction were similar (Table 4; Table 7). Therefore, the classification of the cold tolerance of the varieties was done based on an analysis of the differences in the mean per cent reduction in FGR (in 2010 and 2011 (Table 7). The varieties were classified into four groups depending on their level of cold tolerance as follows: WAB56-104 performed significantly better than other varieties (<20 % reduction) and was classified in group A as very tolerant; reduction did not differ between NERICA 1, 2 and 7 and was by 31 % on average, and hence, these were classified into group B as tolerant; FGR was reduced by 45 % on average in NERICA 3 and 4, and so they were classified in group C as moderately tolerant; and WAB181-18 and NERICA 6, 15 and 16 were classified into group F as susceptible because of their >80 % reduction. The Japanese varieties Koshihikari and Ozora were more affected by cold water irrigation compared with the varieties in group A and B and were grouped separately in group D and E, respectively, while Tsukinohikari was grouped together with the varieties in group F.

Discussion

The evaluation of cold tolerance in NERICAs is important to determine their suitability for production in cold-prone areas such as the East African highlands and also to enable rice breeders to develop efficient breeding programmes to improve rice cultivars. To have stable rice production, especially in high-latitude and high-altitude areas, there is a need to develop rice cultivars with cold tolerance during their growth (Shinada et al. 2013).

The cold tolerance of NERICAs in comparison with their parents and Japanese standard rice varieties was evaluated based on the FGR after cold water irrigation. The FGR differed between the varieties and was greatly reduced by cold water irrigation. Cold stress was more severe in 2009 than in 2011 and was mild in 2010 (Fig. 2; Table 1). The trends in variation for FGR between the varieties were generally consistent in the 3 years except for Koshihikari and NERICA 4 in 2011 (Table 3). Therefore, we used the mean values of 2010 and 2011 to evaluate the cold-stress effect on the reproductive and agronomic characteristics measured. Among the NERICAs, NERICA 1, 2 and 7 showed high FGRs (51.9–57.9 %), while NERICA 6, 15 and 16 showed low FGRs (6.2–14.5 %). NERICA 1, 2 and 7 were less affected by cold stress, with a 31 % mean reduction in FGR, while NERICA 6, 15 and 16 were greatly affected, with their FGR being reduced by more than 80 % (Table 7). NERICA 3 and 4 were moderately affected by cold stress, with about a 45 % reduction in FGR (Table 7). Their grain weights followed similar trends, ranging from 0.1 to 7.5 g/plant (Table 3) and were significantly influenced by FGR, with strong positive correlations (r = 0.83–0.91; $P < 0.001$). This resulted in a grain weights reduction of 61.7–96.4 % under cold stress (Table 5). On the basis of these results, NERICA 1, 2 and 7 showed significantly better performance than NERICA 3 and 4, while NERICA 6, 15 and 16 performed poorly under cold water irrigation. The Japanese varieties Koshihikari and Ozora, classified as

Table 7 Percent reduction rate (%) in filled grain ratio of NERICA and Japanese standard varieties in 2009–2011

Cold tolerance evaluation			Filled grain ratio reduction rate (%)			
Group	Classification	Variety	Mean	2009	2010	2011
A	Very tolerant	WAB56-104	19.3 f		12.0 f	26.6 e
B	Tolerant	NERICA 1	31.7 e	56.4 e	20.2 ef	45.6 cd
B	Tolerant	NERICA 7	30.7 e	54.4 e	21.8 ef	43.1 cd
B	Tolerant	NERICA 2	31.2 e	56.3 e	26.4 ef	37.3 d
C	Moderately tolerant	NERICA 4	41.3 d	60.8 de	44.6 d	38.0 d
C	Moderately tolerant	NERICA 3	47.4 d	76.3 c	43.6 d	50.7 c
D	Moderate	Koshihikari	59.9 c	67.7 cd	27.7 e	92.2 ab
E	Moderately susceptible	Ozora	73.7 b	90.6 ab	61.3 c	86.0 b
F	Susceptible	Tsukinohikari	90.9 a	98.9 a	87.6 a	94.3 ab
F	Susceptible	NERICA 6	89.7 a	99.0 a	88.1 a	91.3 ab
F	Susceptible	WAB181-18	84.4 a		75.7 abc	93.2 ab
F	Susceptible	NERICA 15	85.5 a	99.3 a	66.7 bc	97.9 a
F	Susceptible	NERICA 16	85.3 a	88.7 b	78.0 ab	92.6 ab

Means followed by the same letter within a column are not significantly different (GLM procedure in SAS, $P < 0.05$). Mean indicates average values for 2010 and 2011 data. NERICA, New Rice for Africa very tolerant and moderately tolerant, respectively, were more affected by cold water irrigation than NERICA 1, 2 and 7 (Table 3; Table 7). Similar results of tolerant cultivars performing poorly under cold stress have been reported in Japan (Nakagomi 2013), indicating the need for further improvements in cold-tolerant germplasm and the selection of new check varieties.

WAB56-104 was more tolerant to cold stress than the NERICAs, with high FGR, high grain weight, and low FGR percentage reduction (<20 %), while WAB181-18 was very susceptible, with low FGR, low grain weight and high FGR percentage reduction (84.4 %) in the same range as its progenies, except for NERICA 6 (Table 3; Table 7). WAB56-104 is the *japonica* parent of NERICA 1–11, and WAB181-18 is the parent of NERICA 15–18 (Jones et al. 1997). Therefore, the genotypic differences observed in NERICAs in response to cold are attributed to the genomic contribution from their parents. A high proportion of the *O. sativa* parent genome and high genetic similarities between NERICAs of the same pedigree and their recurrent parent have been reported in NERICAs (Ndjiondjop et al. 2006, Semagn et al. 2006, 2007). However, low genetic similarity has been reported between NERICA 6 and WAB56-104 (Fukuta et al. 2012) and may explain the different performance indicated by NERICA 6 compared with other NERICAs of a similar parent in this study in terms of FGR, plant height, straw dry weight, spikelets per panicle and grain weight (Table 3; Table 5). NERICA 6 has also been classified differently from others by genetic and agronomic trait characterizations (Ndjiondjop et al. 2006, Semagn et al. 2006, 2007). Similar observations have been reported in NERICA 6 yield performance studies under rain-fed conditions (Sekiya et al. 2013). These results reveal that WAB56-104 may contain useful genomic regions associated with cold tolerance, and thus, there is a need for genetic mapping studies to provide this information. This would be useful in marker-assisted breeding programmes for the development of cold-tolerant varieties for production in high-altitude areas, especially in Africa.

On the basis of the mean reduction (%) in FGR under cold stress in 2010 and 2011, WAB56-104 and three NERICA varieties (NERICA 1, 2, and 7) performed better than NERICA 3 and 4, while WAB181-18, NERICA 6, 15 and 16 performed poorly under cold water irrigation (Table 7). From these results, the varieties were classified into four groups referencing several reports (Cruz et al. 2006, Ye et al. 2009, Suh et al. 2010): WAB56-104 in group A as very tolerant (<20 % reduction); NERICA 1, 2 and 7 in group B as tolerant (31 % reduction); NERICA 3 and 4 in group C as moderately tolerant (45 % reduction); and WAB181-18 and NERICA 6, 15 and 16 in group F as susceptible (>80 % reduction). The Japanese varieties Koshihikari and Ozora performed poorly compared to the varieties in groups A and B and were categorized as groups D and E, respectively, while Tsukinohikari was grouped together with the varieties in group F. Therefore, NERICA 1, 2 and 7 are suitable candidates for production in highland regions in East Africa and should be promoted for production. However, further trials are necessary under natural weather conditions in these regions to validate these results, as there may be variations in cold stress intensity, duration and timing.

With regard to the other agronomic traits, the NERICAs were taller and had fewer panicles, but more spikelets per panicle compared to Japanese varieties (Table 4), as reported previously (Matsunami et al. 2009). These traits were also affected by cold stress (Table 5), as reported in other studies (Ye et al. 2009). Low temperature negatively affects plant growth by reducing gas exchange and leaf area expansion (Stuerz et al. 2014a,b) resulting in grain yield losses attributed to reduced spikelet fertility and reduced number of spikelets per panicle (Shimono et al. 2002, Gunawardena et al. 2003b, Stuerz et al. 2014c). Plant height and the number of spikelets per panicle were less affected, while the number of panicles, straw dry weight and grain weights were the traits most affected by cold stress (Table 5). There was reduced growth under cold water irrigation as indicated by the reduction of these agronomic characteristics, especially the high reduction in dry matter accumulation compared with the control (Table 5), which may have indirectly influenced grain-filling ability and grain weights. Most NERICA varieties were less affected in terms of reduction in plant height and straw dry weight compared with the Japanese varieties. Straw dry weights were highly reduced in Koshihikari and WAB56-104. NERICA 6 was least affected by cold stress in plant height and straw dry weight reduction but among the most affected in terms of reduction in spikelet number per panicle and grain weight. NERICA 2, 3, 4 and 7 were most affected in number of panicles reduction but among the least affected in number of spikelets per panicle reduction except for NERICA 7 (Table 5). It was noted that varieties that had greater reduction in the number of panicles were able to maintain their number of spikelets per panicle, which accounted for their high grain weights under cold stress, such as in NERICA 2, 3 and 4. Other studies have also reported the number of spikelets per panicle and spikelet fertility as important yield components that greatly account for big yield differences under cold stress conditions in lowland rice and high-altitude environments in upland rice systems (Shrestha et al. 2012, Stuerz et al. 2014c).

Biomass accumulation at the reproductive stage did not directly contribute to the grain-filling ability and grain yields of the varieties under cold water irrigation. Low correlations were observed between straw dry matter and both

FGR and grain weight, and they were not significant or were either significantly correlated negatively (Table 6). In addition, straw dry matter was significantly higher in NERICA 6 and Tsukinohikari, but these varieties were most affected by cold stress, showing lower FGRs (Table 3). Similarly, the number of panicles and number of spikelets per panicle showed low correlations with FGR under cold stress and were also not significant (Table 6). Nevertheless, the pivotal role of high biomass accumulation in cold tolerance of rice early after transplanting and at the vegetative stages of growth when cold stress occurs early during plant growth has been reported, as it enhances the crop growth rate (Shimono et al. 2002, Arai-Sanoh et al. 2010, Ohsumi et al. 2012). On the other hand, significant positive correlations were also observed between grain weight and number of panicles per plant in 2010 and number of spikelet per panicle in 2011 (Table 6). This means that these traits are also important to maintain grain yields under cold-prone environments. In fact, although NERICA 7 showed higher FGR, its yield was lower under cold stress because of the great reduction in both its number of panicles per plant and the number of spikelets per panicle. In addition, yields of NERICA 3 and 4 were relatively higher than other NERICAs under cold water conditions because of the yield performances under normal water condition and their ability to maintain high number of spikelets per panicle under cold stress. This indicates that we should also consider high-yield traits and not only FGR to progress the breeding on cold tolerance.

Conclusion

This study revealed differences in the cold tolerance ability of NERICA varieties and their parents at the reproductive stage mainly focused on FGR. NERICA 1, 2 and 7 showed higher cold tolerance and were classified as tolerant. NERICA 3 and 4 were classified as moderately tolerant. NERICA 6, 15 and 16 showed higher sensitivity to cold stress and were classified as susceptible. However, NERICA 7 showed lower yields under cold stress and this was attributed to the great reduction in both its number of panicles per plant and the number of spikelets per panicle. Therefore, NERICA 1 and 2 show the potential for good production in high-elevation areas in tropical environments such as the East African highlands. In addition, yields of NERICA 3 and 4 were relatively higher than other NERICAs under cold water conditions because of the yield performances under normal water condition. This indicates that we should also consider high-yield traits and not only FGR to progress the breeding on cold tolerance. In this regard, WAB56-104 had superior performance to all other varieties in terms of yield as well as FGR under cold water conditions, and genetic studies are needed to isolate the chromosomal regions associated with its cold tolerance and provide useful information for marker-assisted breeding. WAB56-104 was identified as a good genetic resource for improvement of the cold tolerance of rice in breeding programmes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 ANOVA for the agronomic characteristics as affected by year and variety, and their interactions under cold stress.