

Identification Of New Sources Of Rice Genotypes (Oryza Sativa L.) Tolerant To Cold Stress

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identification of new sources of rice genotypes (*Oryza sativa* L.) tolerant to cold stress

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Abstract

Rice crop is adversely affected by cold stress which is a common problem in the highland areas of central East Africa (Uganda, Rwanda, north east Tanzania and Kenya) and Madagascar. Cold stress is known to cause 26% to 80% yield loss. Currently, rice production in Uganda is mostly growing to the low altitude areas as there is lack of cold-tolerant varieties that can grow in the higher altitudes of the country. None of the released varieties in Uganda are tolerant cold. The few cold-tolerant lines that have been identified are short-grain types, though Ugandan consumers generally prefer long-grain size. This research aimed at identifying new sources of cold tolerant rice genotypes with preferred traits. A total of 50 lines arranged in a 5x10 alpha lattice design with two replications were used for both stressed and non-stressed experiments. Twenty one (21) days old seedlings were stressed at 10°C in cold air for 10 days and the other remained under normal ambient temperature. IRRI Standard Evaluation System (SES, 2013) was used to score the effect of cold stress on leaf morphology (leaf wilting score) and on leaf color (leaf yellowing score) after 7 day recovery. This study revealed eight highly cold tolerant (SCRID091-20-2-2-4, GIZA 177, NERICA 1, MET P27, MET P23, MET P60, MET P20 and MET P5) in which only GIZA 177 was short grain and fifteen tolerant varieties (MET P32, MET P11, MET P2, MET P17, MET P3, MET P24, MET P16, MET P37, MET P18, MET P9, MET P40, MET P31, MET P39, MET P22, MET P36) were identified.

Key words: Rice crop, Seedling growth, cold stress

3.1 Introduction

Rice is a sensitive crop that frequently exposed to variable biotic and abiotic stress which adversely affect growth and production (GRiSP, 2013). Biotic constraints include; weeds, blast, rice yellow mottle virus (RYMV) and African rice gall midge (AfRGM). Major abiotic constraints include; extreme temperatures (heat and cold) (Dramé *et al.*, 2013; Zhang *et al.*, 2014), flood and drought (Odogola, 2006 ; Namazzi *et al.*, 2010), and variable rainfall (Akongo *et al.*, 2016) as well as soil problems (salinity, nutrient deficiencies and toxicities) (NEWEST, 2012; Dramé *et al.*, 2013). However, cold stress is the most devastating abiotic production constraint which affects the rice plant at all growth stages from germination to grain filling, leading to high economic yield losses (Gothandam, 2012, Shinada *et al.*, 2013). It is observed in the high altitude areas around the globe. Cold stress that occur at critical reproductive stages can adversely affect grain quality and cause yield reductions in high-altitude regions of the world (Singh *et al.*, 2017). The loss in quality and quantity is as a result of severe reduction in seedling establishment, growth retardation, decrease tiller number, delay and incomplete grain maturation (Satake and Hayase, 1974).

In Uganda, identification of rice genotypes tolerant to cold stress is still in its exploratory stage and all the varieties under production are sensitive to the stress. Some cold tolerant rice genotypes were identified by Nyiramugisha *et al.* (2016) but they were short grained, sticky and non-aromatic; characteristics which is not preferred by farmers. In this study, the susceptibility and tolerance of 50 rice genotypes to cold stress was investigated. The aim was to identify rice genotypes with new sources of tolerant to cold stress and with acceptable grain size for the improvement of rice in current breeding programme.

3.2 Materials and Methods

3.2.1 Genetic materials and description of study area

Fifty (50) genotypes, including a tolerant and a susceptible check, were obtained from NaCRRI, in which their sources were from: Africa Rice (Ibadan, Nigeria), African rice (Benin), Egypt, Tanzania and Madagascar were used for the study (Table 1). The study was conducted at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) (0°28'N and

32°37'E, approximately 1200 masl). The area receives a mean annual rainfall of 1200 mm with an average temperature ranging between 17°C and 33°C. Screening was conducted in an artificially lighted room under controlled temperature of 10°C, while the temperature of the screen house used to grow the control experiment was between 23°C and 27°C.

Table 1: Rice genotypes evaluated for cold tolerance at seedling stage.

No.	Pedigree	Line Name	Source	Other information	Grain size
1	ART35-200-2-2-B-1	MET P10	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
2	ART27-190-1-3-3-1	MET P27	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
3	ART35-4-1-5D-1	MET P9	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
4	ARS126-3-B-1-2 (11)	ARS126-3-B-1-2 (11)	Africa Rice-Benin,		Long
5	ART34-79-1-2N-2	MET P5	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
6	ART27-58-7-1-2-4-2-2	MET P21	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
7	ART27-190-6-4-2-1-1	MET P19	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
8	ART34-88-1-2-B-1	MET P12	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
9	ART27-122-19-3-1-3	MET P37	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
10	ART16-5-9-22-3-B-B-2	MET P38	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
11	ART35-114-1-6N-2	MET P3	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
12	ARC36-4-ET-2 (3)	ARC36-4-ET-2(3)	Africa Rice-Benin,		Long
13	ART35-52-2-7N-2	MET P1	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
14	ART27-58-6-2-1-1-3-3	MET P30	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
15	ART27-58-8-1-2-3	MET P29	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
16	ART34-82-1-7N-1	MET P2	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
17	ART27-190-7-3-2-4-3-1	MET P32	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
18	ART27-190-6-1-4-2-2-1	MET P26	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
19	ART34-86-2-1-B-1	MET P11	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
20	ART35-49-1-4N-1 /MET 6	MET P6	Africa Rice-Ibadan, Nigeria	<i>O.barthi interspecific lines</i>	Long
21	ART34-256-3-1-B-2	MET P14	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
22	ARC39-145-P-2 (5)	ARC39-145-P-2 (5)	Africa Rice-Benin,		Long
23	ART27-190-1-4-2-1-1-3	MET P40	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
24	ART27-58-7-2-2-3	MET P20	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
25	ART27-58-6-2-2-2	MET P28	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
26	ARC36-2-P-2-54 (2)		Africa Rice-Benin,		Long
27	ART27-58-6-2-1-1-3-1	MET P33	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
28	ART27-122-19-3-1-2-1-1	MET P36	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
29	NERICA 1	NERICA 1	Africa Rice	Cold tolerant (Check)	Long
30	ART27-58-7-1-2-2-2-2	MET P17	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long

31	ART34-76-2-8D-2	MET P7	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
32	ART34-113-3-2-B-1	MET P13	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
33	ART27-58-3-2-1-1	MET P22	Africa Rice	<i>O.barthi</i> interspecific lines	Long
34	WITA 9	TOX 3058-28-1-1-1	Africa Rice	Susceptible to cold(check)	Long
35	PCT-4\0\0\1>295-2-3-1-3-3-M	MET P60	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
36	ART27-190-7-6-4-2	MET P39	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
37	ARC36-2-1-2	ARC36-2-1-2	Africa Rice-Benin,		Long
38	ART15-7-16-38-1-B-B-2	MET P31	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
39	ART35-100-1-7D-1	MET P8	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
40	ART27-58-6-2-1-1-3-2	MET P25	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
41	ART27-58-3-2-2-1	MET P35	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
42	ART3-7L9P8-3-B-B-2-1	MET P24	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
43	ART27-58-8-1-1-4	MET P23	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
44	ART27-58-6-2-1-3	MET P34	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
45	ART27-58-3-2-1-4	MET P18	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
46	ART35-272-1-2-B-1	MET P16	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
47	GIZA 177	GIZA 177	Egypt	Japonica subs pecieses	Short
48	ART35-159-1-2-B-1	MET P15	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long
49	SCRID091-20-2-2-4-4	SCRID091-20-2-2-4-4	Tanzania	Origin Madagascar	Medium
50	ART34-146-1-8N-1	MET P4	Africa Rice-Ibadan, Nigeria	<i>O.barthi</i> interspecific lines	Long

3.2.2 Experimental design and data collection

A total of fifty genotypes were tested for their reaction to cold stress following the protocol described by (Lee, 2001). Seedlings of 3-4 leaf stage (21 days after planting) were exposed to 12°C/10°C (day/night) cold air temperature for 10 days in a cold growth chamber following a 12/12 hour day/night artificial lighting cycle. Ten days after stress treatment, the seedlings were taken back to normal growth conditions to allow the genotypes to recover, for seven days. The experiments were laid out in a 5 by 10 alpha lattice design, replicated twice. Two sets of experiments were established with one group exposed to cold stress while the other group was made to grow at normal temperature conditions (stress free). The seeds were planted in bin plastic pots of 24 cm x 36 cm width filled with forest soil. Each genotype was planted in a pot containing twelve hills.. Di-ammonium phosphate (DAP) fertilizers (18-46-0) was applied at the rate of 0.4 g/pot during planting while urea (46-0-0) was top dressed at the rate of 0.4 g/pot fifty days after germination. All agronomic management practices were done regularly as needed. Data based on visual rating (leaf growth/leaf wilting score and seedling color/leaf yellowing score) using Standard Evaluation System (SES) (IRRI, 2013), survival rate, seedling height and tiller number were collected 7 days after recovery for the stressed plants. Similar data were also collected from the non-stressed plants. However, for the non-stressed experiment, genotypes were not different for visual rating data and survival rate. As a result these parameters were not considered for further analyses. Instead, seedling height, tiller number and grain yield per pot were used for comparison. Survival rate was calculated using the following formula from (Nohrman, 1953), i.e.

$$\text{Survival rate(\%)} = \frac{\text{Surviving plants}}{\text{Total number of treated plants}} * 100$$

Seedling color and leaf growth were taken based on scale of 1-9 developed by IRRI (2013). The scales for these two traits were basically given by the extent of cold damage on leaf coloration and leaf morphology respectively. For seedling color, a score of 1 represents dark green seedling while a score of 9 represents dead seedling. For leaf growth, a score of 1 represents no damage on leaf and normal leaf color and hence highly tolerant while 9 represents most seedlings are dead or dying and hence highly sensitive. Therefore, based on leaf growth damage, seedling with

scores 1-3 are considered as cold tolerant, 4-6 are intermediate and 7-9 are susceptible. The detailed description for the scales is presented in Table 2.

Table 2: Description of seedling color and leaf growth under cold stress

Trait	Scale	Description
Seedling color	1	Dark green
	3	Light Green
	5	Yellowish
	7	Brown
	9	Dead
Leaf growth	0-1	No damage of leaf normal leaf color (high tolerance)
	2-3	Tip of leaves slightly dried, folded (tolerant)
	4-5	Some seedlings moderately folded and wilted
	6-7	Seedlings severely rolled and dried; reddish-brown leaves
	8-9	Most seedlings dead and dying or highly sensitive

Scaling and description according to IRRI, (2013)

3.2.3 Data analysis

Data were analyzed using GenStat software, 18th edition (Payne *et al.*, 2015) Restricted Maximum Likelihood (ReML) approach was used to generate analysis of variance (ANOVA) and to examine differences in the performance of different rice genotypes for their tolerance to cold stress. Fisher's LSD test was used to separate the means. Pearson correlation was used to examine the association among the different tolerance parameters. The model used was as follows;

$$Y_{ijk} = \bar{Y} \dots + G_i + R_j + B_{kj} + E_{ijk}$$

Where, Y_{ijk} = The observed value of trait from the i^{th} genotype from the k^{th} block nested in the j^{th} replicate, $\bar{Y} \dots$ = Grand mean, G_i = the effect of i^{th} genotype, R_j =Effect of j^{th} replication,

$B_{k(j)}$ is the effect of the k^{th} block nested within the j^{th} replicate, E_{ijk} = random residual or error term

Base index was estimated by the linear combination of the mean phenotypic values of the traits weighted directly by their respective economic weights (Williams 1962). Base index value was calculated as:

$$I = a_1y_1 + a_2y_2 \dots \dots a_ny_n = \sum_{i=1}^n a_iy_i$$

Where, y =Mean values, a = is economic weight of the economic characters under study

Tolerance indices and reduction percentage were calculated for yield per pot for the data collected from stress and non-stress experiment. Cold tolerance indices were calculated as follow;

$$SSI = (1 - (Y_s/Y_p)) / (1 - (\bar{Y}_s/\bar{Y}_p)) \dots \dots (\text{Fischer and Maurer, 1978})$$

$$TOL = Y_p - Y_s \dots \dots (\text{Rosielle and Hamblin, 1981})$$

$$YSI = Y_s/Y_p \dots \dots (\text{Bousslama and Schapaugh, 1984})$$

$$\text{Reduction (\%)} = \left(\frac{Y_p - Y_s}{Y_p} \right) * 100 \dots \dots (\text{Choukan et al., 2006})$$

Where Y_s = yield under stress, Y_p = Yield under non- stress, \bar{Y}_s =Yield grand mean under stress, \bar{Y}_p =Yield grand mean under non-stress, SSI= stress susceptibility index, TOI=Tolerance index

3.3 Results

3.3.1 Response of rice genotypes to cold stress

The analysis of variance revealed a highly significant difference ($p < 0.001$) among genotypes for traits collected under stress (leaf growth, seedling color, survival rate, seedling height and tiller number) (Table 3), indicating genetic variability among tested genotypes in their reaction to cold stress. However, the genotypes did not show significant difference under non-stress conditions to all the traits except seedling height significant at $p < 0.01$ (Table 4).

Table 3: Mean square for rice genotypes screened at seedling stage at 10°C

Source of variation	¹ Df	² LG	³ SC	⁴ SR	⁵ SH	⁶ TN	⁷ GY(g)/pot	% reduction SH	% reduction TN
Replication	1	0.01 ^{ns}	0.14 ^{ns}	831.38 ^{***}	2.4 ^{ns}	0.5 ^{**}	2197.30 ^{**}	768.2 ^{***}	1806.2 ^{***}
Block/Replication	8	0.48 [*]	0.80 ^{**}		6.7 ^{ns}	0.14 ^{ns}	192.50 ^{ns}	95.5 ^{***}	294.4 ^{***}
Genotype	49	10.7 ^{**}	11.51 ^{***}	1574.93 ^{***}	97.4 ^{***}	0.7 ^{***}	434.31 ^{**}	544.9 ^{***}	942.8 ^{***}
Error	41	0.20	0.26	63.51	5.6	0.13	140.90	53.67	131.3
⁸ LEE	25-30	0.23	0.36		5.9	0.14	158.51	64.0	165.1
⁹ SED		0.5	0.6	7.96	2.4	0.37	12.59	8.0	12.9
¹⁰ CV (%)		12.05	14.3	10.7	9.6	19.8	24.2	22.9	35.5

*, **, *** = significant at 0.05, 0.01 and 0.001 respectively, ns = non-significant, ¹Degree of freedom ²Leaf growth, ³Seedling color, ⁴Survival rate, ⁵Seedling height, ⁶Tiller number, ⁷Grain yield in gram, ⁸Lattice effective error, ⁹Standard error of Difference, ¹⁰Coefficient of variation

Table 4: Mean square of rice genotypes under non- stress condition

Source of variation	¹ Df	² SH	³ TN	⁴ GY(g)/pot
Replication	1	368.1***	3.93 ^{ns}	24.40 ^{ns}
Block/Replication	8	16.1	0.96 ^{ns}	437.40 ^{ns}
Genotype	49	36.05**	1.13 ^{ns}	213.50 ^{ns}
Error	41	12.37	0.56	213.70
⁵ LEE	27-31	13.42	0.79	257.28
⁶SED		3.66	0.89	16.04
⁷CV (%)		9.53	29.79	21.4

*** = significant 0.01, ns = non-significant, ¹Degree of freedom, ²Seedling height, ³Tiller number, ⁴Grain yield in gram, ⁵Lattice effective Error, ⁶Standard error of difference, ⁷Coefficient of variation

3.3.2 Performance of rice genotypes under stress and non-stress conditions

The tested genotypes were classified into five different cold tolerance categories based on the (IRRI) (SES) scale with a little modification using leaf growth/leaf wilting score. Eight genotypes recorded score ranging from 1.0 to 1.3 were classified as highly tolerant, fifteen tolerant were classified as tolerant (score 1.7-3.1), nine were classified as moderately tolerant (score 3.2-4.8), thirteen were classified as susceptible (scores 5.5-6.6) and five classified as highly susceptible (7.6-9.0) (Table 5, Figure 1 and Appendix 1).

Table 5: Classification of rice genotypes based on their level of cold tolerance using leaf growth scale

Category	Genotypes	Numbers
Very tolerant (TT)	MET P27, GIZA 177, MET P23, SCRID091-20-2-2-4-4, MET P20, NERICA 1, MET P60, MET P5	8
Tolerant (T)	MET P32, MET P11, MET P2, MET P17, MET P3, MET P24, MET P16, MET P37, MET P18, MET P9, MET P40, MET P31, MET P39, MET P22, MET P36	15
Moderate tolerant (MT)	MET P34, MET P4, MET P8, MET P29, MET P35, MET P15, MET P28, MET P19, MET P25	9
Susceptible (S)	MET P1, ARC36-2-P-2-54 (2), MET P13, MET P7, MET P6, MET P30, MET P12, MET P33, MET P10, MET P26, MET P38, MET P14, MET P21	13
Highly susceptible (HS)	WITA 9, ARC36-2-1-2, ARC36-4-ET-2 (3), ARC39-145-P-2 (5), ARS126-3-B-1-2 (11)	5

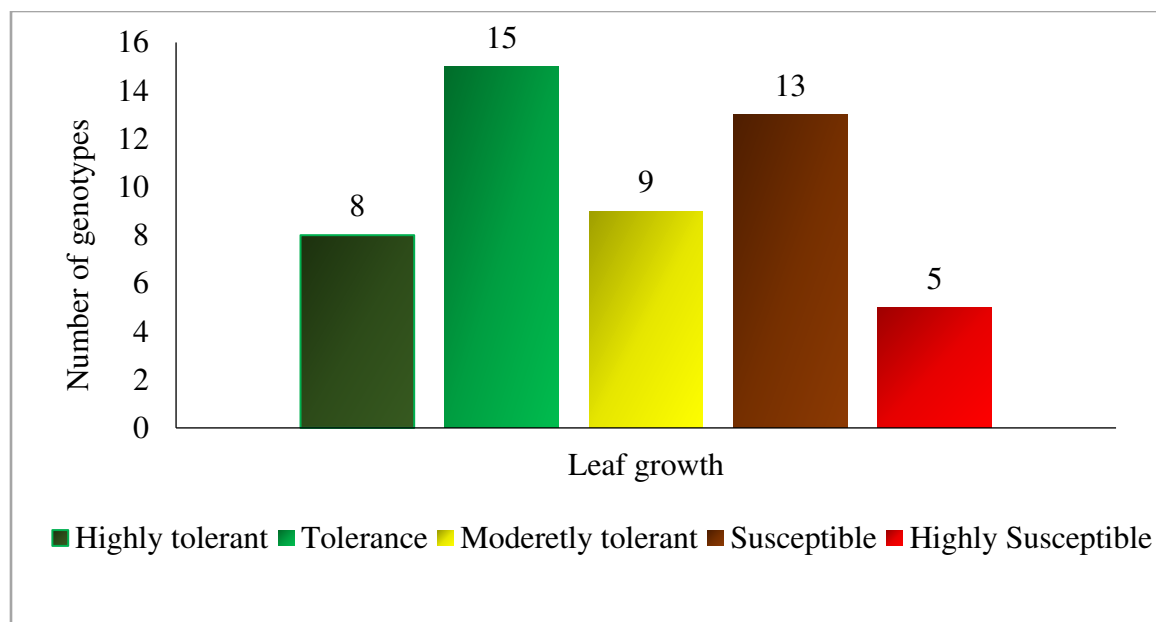


Figure 1: Reaction categories of 50 rice genotypes to cold stress at seedling stage in terms leaf wilting score after recovery

Seedling color score varied significantly ($p < 0.001$) among the tested genotypes after stress treatment recovery (Table 3). The seedling of the tested genotypes fell into five color categories. Five genotypes exhibited a dark green color with the mean score ranging from 1.0 to 1.39, eighteen were light green (mean score ranging 1.6-2.5), ten yellowish (mean score ranging 3.6-5.4), thirteen brownish (mean score ranging 5.6-6.9) while four seedlings dried and died (mean score ranging 8.3-9.1) (Figure 2 and Appendix 1). Genotypes ARC39-145-P-2 (5) and (ARS126-3-B-1-2 (11) which were the most susceptible were completely dead after recovery.

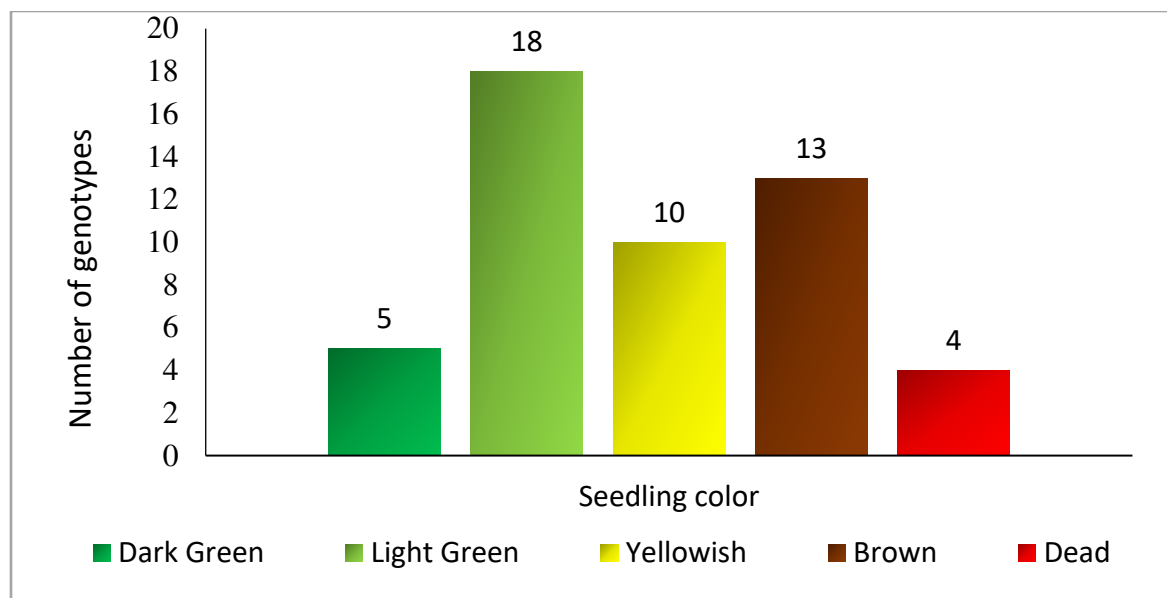


Figure 2 : Reaction categories of 50 rice genotypes to cold stress at seedling stage in terms of leaf yellowing score after recovery

Survival rate results showed a significant variation among the tested genotypes after stress recovery. It varied from 0% (completely dead) to 100% (complete seedlings survival). Highly cold tolerant genotypes exhibited 100% survival rate whereas the highly susceptible genotypes revealed low survival rate (0-33%) (Appendix 1). The cold tolerant check (GIZA177) exhibited low score (little damage on leaf and dark green color) and high survival rate, whereas susceptible check TOX 3058-28-1-1-1/WITA 9 showed higher Score (most seedling dried and reddish brown in color) and low survival rate (Figure 3)

The evaluated genotypes varied significantly in their seedling heights both under stress and non-stress conditions (Table 3 and Table 4). Seedling height under stress ranged from nothing to 37.2 cm while that under non-stress conditions ranged from 24.9 cm to 45.1 cm (Appendix 1). The tallest genotype under stress was SCRID091-20-2-2-4-4 (37.2 cm) followed by MET P9 (34.1 cm) while the shortest genotype was ARC36-2-1-2 (8.5 cm). Likewise, genotypes MET P9 and SCRID091-20-2-2-4-4 were the tallest under non-stress (45 cm each) (Appendix 1). In general, lower seedling heights were recorded from the stressed plants compared to the non-stressed plants (Appendix 1). Genotypes varied significantly in their reduction in seedling heights. Genotypes MET P23, MET P27, MET P 60 SCRID091-20-2-2-4-4 and NERICA 1 had the least reduction in seedling height with reduction of less than 20%. Genotypes ARS126-3-B-1-2 (11)

and ARC39-145-P-2 (5) were the least tolerant to cold stress with 100% yield reduction implying that the crop was completely destroyed under cold stress (Appendix 1).

The number of tillers per hill varied significantly for genotypes evaluated under cold stress (Table 3). However, there was no significant difference among genotypes for tiller number under non-stress conditions. The number of tiller per hill ranged from 1 to 3 for stressed genotypes and 2 to 5 for non-stressed genotypes. The highest numbers of tillers were recorded for MET P60, followed by MET P27 GIZA 177, NERICA 1 and SCRID091-20-2-2-4-4, while lowest tiller number was recorded for ARC36-2-1-2 in the stressed seedlings. Reduction in tiller number for non-stress and stress condition varied significantly for the evaluated genotypes (Table 3). Genotypes MET P22, MET P60, MET P27, MET P20, MET P40, NERICA 1 and SCRID091-20-2-2-4-4 had the least reduction in tiller number with reduction of less than or equal to 20%. The highest reduction was recorded for genotype ARS126-3-B-1-2 (11) and ARC36-2-1-2 in which cold stress caused 100% reduction (Appendix 1).

Base index was employed to select top cold tolerant genotypes for the five main tolerance traits i.e. seedling color (SC), leaf growth (LG), survival rate (SR), tiller number (TN) and seedling height (SH). Based on the sum base index value, the best performing genotype was SCRID091-20-2-2-4-4 (128.0) followed MET P27 (125.9) and MET P60 (124.8). The least performing genotype was ARS126-3-B-1-2 (11) (Table 6).

Table 6: Mean performance on main traits with base index rank of 50 rice genotypes evaluated at seedling stage in cold growth chamber

Line name	¹ LG	² SC	³ SH	⁴ TN	⁵ SR	Base index
SCRID091-20-2-2-4-4	1.2	1.4	37.2	2.6	100.0	128.0
MET P27	0.9	1.1	33.4	2.9	100.0	125.9
MET P60	1.3	1.7	33.1	3.0	100.0	124.8
MET 23	1.1	1.0	32.1	2.2	100.0	124.1
GIZA 177	1.0	0.7	30.2	2.8	100.0	123.7
MET P9	2.6	2.5	34.1	2.4	100.0	122.8
MET P40	2.6	2.6	32.5	2.2	100.0	121.5
MET P5	1.3	1.5	29.0	2.3	100.0	121.1
NERICA 1	1.3	2.2	29.1	2.7	100.0	121.1
MET P32	1.7	1.7	29.9	1.7	100.0	120.8
MET P20	1.2	1.4	27.6	2.2	100.0	120.2
MET P11	1.8	1.8	26.8	2.1	100.0	118.7

MET P17	1.8	2.2	27.0	2.0	100.0	118.2
MET P2	1.8	1.9	25.6	2.3	100.0	117.9
MET P22	3.0	2.7	26.6	2.9	100.0	117.2
MET P24	2.1	2.2	26.0	2.0	100.0	117.2
MET P16	2.2	3.6	28.0	1.7	100.0	116.9
MET P36	3.1	1.8	26.1	2.0	100.0	116.7
MET P3	2.1	2.5	26.1	1.6	100.0	116.6
MET P37	2.5	2.7	29.7	1.9	95.8	114.8
MET P39	2.9	2.8	23.8	2.1	100.0	114.3
MET P18	2.5	3.4	25.2	2.3	95.9	111.1
MET P31	2.9	3.0	28.6	2.6	79.2	97.4
MET P4	3.5	3.9	23.6	1.5	79.2	91.0
MET P8	3.6	4.3	22.8	2.4	79.2	90.8
MET P33	6.2	4.5	26.2	1.7	79.2	89.8
MET P6	5.9	5.5	26.0	1.2	75.0	84.3
MET P35	3.9	4.1	25.0	1.4	70.8	83.0
MET P30	6.0	5.1	23.1	1.4	70.8	78.4
MET P25	4.8	3.8	28.4	2.0	62.5	77.2
MET P28	4.1	5.1	28.9	2.0	62.5	77.0
MET P15	4.1	6.1	27.0	2.2	62.5	74.7
MET P34	3.2	3.4	24.5	2.1	60.0	73.8
MET P7	5.8	5.8	26.4	1.3	62.5	72.1
MET P26	6.4	5.6	25.1	1.7	62.5	70.9
MET P14	6.5	6.5	25.1	1.9	62.5	70.3
MET P19	4.3	6.1	26.0	2.0	58.3	69.5
MET P38	6.5	5.5	25.8	1.8	58.3	67.5
MET P12	6.2	6.2	26.4	1.4	58.3	67.1
MET P21	6.6	6.7	25.1	1.3	58.3	65.3
MET P13	5.7	6.2	22.3	1.7	58.3	64.8
MET P1	5.5	5.6	25.1	1.3	54.2	63.2
MET P10	6.3	5.8	25.8	1.6	54.2	63.0
MET P29	3.8	5.9	25.6	1.8	50.0	61.3
WITA 9	7.5	6.9	17.4	1.8	33.3	33.7
ARC36-2-P-2-54 (2)	5.7	6.9	16.9	1.4	29.2	30.7
ARC36-4-ET-2 (3)	8.7	8.7	14.7	0.6	29.2	23.4
ARC36-2-1-2	7.7	8.3	8.5	0.5	12.5	3.4
ARC39-145-P-2 (5)	9.0	9.1	0.1	1.0	0.0	-17.0
ARS126-3-B-1-2 (11)	9.0	9.0	0.1	0.0	0.0	-17.9
Mean	4.0	4.2	25.2	1.9	74.3	
⁶LSD (5%)	0.9	1.2	5.0	0.8	16	

¹Leaf growth, ²Seedling color, ³Seedling height, ⁴Tiller number, ⁵Survival rate and ⁶Least Significant Difference

3.3.3 Relationship between and among traits evaluated in the stressed experiment

Leaf growth showed negative highly significant correlation with seedling height ($r = -0.70^{***}$), survival rate ($r = -0.87^{***}$), tiller number ($r = -0.75^{***}$) and grain yield (g) ($r = -0.83^{***}$) but was positively correlated with seed color ($r = 0.93^{***}$). Seedling color showed negative highly significant correlation with seedling height ($r = -0.73^{***}$), survival rate ($r = -0.93^{***}$), tiller number ($r = -0.74^{***}$) and grain yield ($r = -0.78^{***}$). Seedling height, survival rate and tiller number, showed strong positive association with grain yield at $r = 0.66^{***}$, 0.75^{***} and 0.67^{***} , respectively (Table 7).

Table 7: Pearson correlation coefficients between morphological parameters (n=50) in 50 rice genotypes exposed to cold stress of 10°C at seedling stage

	¹ LG	² SC	³ SH	⁴ SR	⁵ TN
¹ LG					
² SC	0.93 ^{***}				
³ SH	-0.70 ^{***}	-0.73 ^{***}			
⁴ SR	-0.87 ^{***}	-0.93 ^{***}	0.75 ^{***}		
⁵ TN	-0.75 ^{***}	-0.74 ^{***}	0.70 ^{***}	0.69 ^{***}	
⁶ GY(g)/pot	-0.83 ^{***}	-0.78 ^{***}	0.66 ^{***}	0.75 ^{***}	0.67 ^{***}

^{***}, = significant at ($p < 0.001$), ¹Leaf Growth ²Seedling Color, ³Seedling Height, ⁴Survival Rate ⁵Tiller number, ⁶Grain yield in gram

3.3.4 Comparison of rice genotypes based on tolerance indices

Performance differences between stressed and non-stressed genotypes were clearly seen after recovery and in later growth stages (Figure 3).



Figure 3: Effect of cold stress on rice seedlings after recovery and in later growth stage. Damage due to cold stress as observed (drying, yellowing and stunting). A and C are non-stressed and B and D are cold stressed

Stress tolerance indices were calculated for grain yield from genotypes in the stressed and non-stressed treatments. Predicted mean of yield under stress (Y_s), predicted mean of yield under non-stress (Y_p), reduction percentage, stress susceptibility index (SSI), tolerance index (TOL) and yield stability index (YSI), are presented in Table 8. The reduction rate ranged from 7.33 to 100% for grain yield. The lowest reduction in grain yield was recorded for genotype SCRID091-20-2-2-4-4 (7.33%) followed by MET P20 (7.97%), indicating tolerance to cold stress. Highest yield reduction percentage was recorded for ARC39-145-P-2 (5) and ARS126-3-B-1-2 (11) at 100%, suggesting high susceptibility to cold stress. The SSI value ranged from 0.24 to 3.29. The lower the SSI value the more tolerant the genotypes is. The lowest SSI value, was calculated for

genotype SCRID091-20-2-2-4-4 (0.24), followed by MET P20 (0.26) and MET P27 (0.27) indicating that these genotypes were cold tolerant. On the other hand, a high SSI value (3.29) was calculated for ARS126-3-B-1-2 (11) and ARC39-145-P-2 (5) suggesting that these two genotypes are the most susceptible to cold stress. TOL value ranged from 4.35 to 76.26g. The lower TOL value (yield difference) was observed for genotype MET P27 (4.35g) followed by MET P20 (5.47g) and SCRID091-20-2-2-4-4 (5.68g). The Highest yield differences between stress and non-stress was observed for genotype ARC39-145-P-2 (5) (76.26g), suggesting susceptibility to cold stress. YSI, which is the ratio of yield under stress to non-stress ranged from 0 (ARS126-3-B-1-2 (11) and ARC39-145-P-2 (5)) to 0.93 (SCRID091-20-2-2-4-4) (Table 8 and Appendix 2).

Table 8: Tolerance indices of the best 23 cold tolerance and four highly susceptible rice genotypes under stress and non-stress conditions for grain yield

Line name	GY (g)/pot (stressed)	GY (g)/pot (Non-stressed)	Reduction (%)	SSI	TOI	YSI
SCRID091-20-2-2-4-4	71.8	77.5	7.3	0.2	5.7	0.9
MET P20	63.1	68.6	8.0	0.3	5.5	0.9
MET P27	49.4	53.7	8.1	0.3	4.4	0.9
MET P5	73.0	79.6	8.2	0.3	6.5	0.9
GIZA 177	64.8	72.0	9.9	0.3	7.2	0.9
MET P60	61.8	69.4	11.0	0.4	7.6	0.9
NERICA 1	85.9	97.1	11.5	0.4	11.2	0.9
MET P40	49.7	56.4	12.0	0.4	6.7	0.9
MET P23	69.0	79.0	12.7	0.4	10.0	0.9
MET P3	57.6	66.5	13.3	0.4	8.8	0.9
MET P18	76.3	88.1	13.4	0.4	11.8	0.9
MET P2	63.4	74.2	14.5	0.5	10.8	0.9
MET P32	63.7	74.9	15.0	0.5	11.2	0.9
MET P37	53.9	64.1	15.9	0.5	10.2	0.8
MET P11	72.9	88.6	17.7	0.6	15.7	0.8
MET P17	65.0	79.1	17.8	0.6	14.1	0.8
MET P9	66.3	81.4	18.5	0.6	15.1	0.8
MET P16	49.8	61.6	19.2	0.6	11.9	0.8
MET P36	68.4	85.6	20.1	0.7	17.2	0.8
MET P39	46.1	57.8	20.3	0.7	11.7	0.8
MET P31	61.5	77.6	20.7	0.7	16.1	0.8
MET P22	54.9	69.7	21.2	0.7	14.8	0.8
MET P24	56.6	73.5	22.9	0.8	16.9	0.8

ARC36-2-1-2	30.2	83.0	63.7	2.1	52.8	0.4
ARC36-4-ET-2 (3)	11.6	63.1	81.7	2.7	51.6	0.2
ARS126-3-B-1-2 (11)	0.0	71.4	100.0	3.3	71.4	0.0
ARC39-145-P-2 (5)	0	76.3	100.0	3.3	76.3	0.0
Mean	52.1	74.8				
LSD (5%)	29.1	Ns				

GY (g) = (Grain Yield in gram), TOL=Tolerance index, SSI= Stress Susceptibility Index, YSI= Yield Susceptibility Index LSD= Least Significant Difference and ns=non-significant

3.4 Discussion

The study demonstrates the existence of new sources of tolerance to cold stress in the new rice genotypes which could be used to introgress cold tolerance into farmers' preferred but cold susceptible rice cultivars. Substantial variations were observed among the tested rice genotypes for cold stress parameters such as leaf growth/leaf wilting and seedling color/leaf yellowing, survival rate, tiller number and seedling height (Table 5).

Leaf growth/leaf wilting score was able to distinguish the tolerant and susceptible genotypes. Similarly, Andaya and Mackill (2003), were able to identify highly cold tolerant rice genotypes at seedling stage using leaf growth/leaf wilting score and suggested that leaf growth could be the most suitable parameter in evaluating cold tolerance at seedling stage. The evaluated genotypes were grouped into five categories based on their reaction to cold treatments. The categories include; highly tolerant (8 genotypes), tolerant (15 genotypes), moderately tolerant (9 genotypes), susceptible (13 genotypes) and highly susceptible (5 genotypes). Of the 8 highly tolerant genotypes 5 genotypes i.e. MET P27, MET P23, MET P20, MET P60 and MET P5 were newly identified while the other 3 genotypes i.e. GIZA177, SCRID091-20-2-2-4-4 and NERICA 1 had previously been reported show tolerance to cold stress. SCRID091-20-2-2-4-4 by Nyiramugisha *et al.* (2017), GIZA 177 by Suh *et al.* (2013) and NERICA 1 by Dessie *et al.* (2014) and Wainaina *et al.* (2015). In addition, GIZA 177 is a Japonica subspecies. Japonica subspecies are known to be cold tolerant as confirmed by Cruz and Milach (2004) and Park *et al.* (2013). SCRID091-20-2-2-4-4 has background of origin of Madagascar, which is one of the places where rice is grown mostly in the highland parts of the country. The newly identified highly cold tolerant genotypes were from African rice Ibadan Nigeria and they were from the *O.barthi* interspecific line. Even though, GIZA 177 is a highly cold tolerant genotype, it is of a short grain type which is not preferred by Ugandan farmer. However, it can be a good source of cold tolerance for breeding programme improving farmer preferred varieties. SCRID091-20-2-2-4-4 is a medium grain size variety. The other newly identified genotypes (i.e. MET P27, MET P23, MET P60, MET P20 and MET P5) have long grain sizes preferred by Ugandan farmers. Leaf growth/leaf wilting scores were significantly ($p < 0.001$) and negatively correlated survival rate, tiller number, seedling height and grain yield (g) per pot (Table 9), suggesting the lower the score the better performance of genotypes. There was strong positive correlation between leaf

growth and seedling color scores ($r = 0.93^{***}$) suggesting that selection can be done using any of the traits.

The loss of chlorophyll content observed in rice seedlings was evaluated using seedling color/leaf yellowing scores. The tested genotypes were grouped into five different leaf color categories based on seedling color observed after recovery. The categories included dark green (with 5 genotypes), light green (with 18 genotypes), yellowish (with 10 genotypes), brownish (with 13 genotypes) and mostly dried and dead seedlings (with 4 genotypes). Cold stress heavily affects chlorophyll content, mainly exhibited through leaf discoloration which finally weakens the photosynthetic ability of the seedling (Lou *et al.*, 2007; Kim *et al.*, 2012; Pradhan and Rani, 2017). Studies conducted by Kim *et al.* (2012) and Donoso *et al.* (2015) confirmed that seedling color scores was related to chlorophyll content. In this study, seedling color was significantly and negatively correlated with survival rate, seedling height, tiller number and finally grain yield. This suggested that seedlings with green color (i.e lower score) had good survival, height, tiller and grain yield. In other words, genotypes with lower seedling color scores had better photosynthetic ability than seedlings with higher scores. Similarly, Ranawake and Nakamura (2011) reported that genotypes with green color exhibited high height in their evaluation of inbred lines for cold tolerance at seedling stage.

Assessment of survival rate was effective in identifying degree of recovery in genotypes. Cold tolerant genotypes recovered up to 100%, whereas susceptible genotypes were unable to recover and/or had low recovery from the applied cold stress. These results were similar to Kim *et al.* (2012) in their study conducted to evaluate rice seedlings tolerance to constant and intermittent low temperature stress observed good recovery. Survival rate was significantly and positively correlated with seedling height, tiller number and grain yield per pot. The higher survival rate the more tolerant the genotypes and the better the performance in later growth.

Seedlings were stunted under applied cold treatment. This was clearly observed by comparing seedlings under stress and non-stressed conditions. Seedling height under stress ranged from 8.51 to 37.2 cm and 24.9 to 45.2 cm for those in non-stress conditions. Stunting at seedling stage is of the major indicators of cold stress, as reported by Yoshida (1981); Pradhan *et al.* (2017) and Zhang *et al.* (2014). In this study, seedling height was positively correlated with survival rate, tiller number and grain yield per pot.

Examination of tillering is one useful way to examine the growth status of rice plants (Yoshida, 1981). In this study, tiller number per hill was ranged from 1 to 3 and 2 to 5 for the stressed and non-stressed experiment respectively. Lower tiller number was exhibited under stressed conditions suggesting that cold stress affected tillering ability. Similar result were obtained Shimono *et al.* (2007) and Ndour *et al.* (2016) who reported that low temperature at seedling stage reduced tillering ability.

Twenty three best cold tolerant rice genotypes were selected by base index selection using five traits, for consistent performance under highland parts of Uganda. Base index is a method of selection where indices are established by the linear combination of the mean phenotypic values of the characters weighted directly by their respective economic weights (Williams, 1962). Economic weight was given to the traits based on their importance to identify cold tolerance. As Biosci *et al.* (2013) reported economic weight is given to certain traits based on breeding objectives and their importance or contribution in selecting genotypes of interest. Traits with high genetic variance had the power to distinguish tolerance and susceptibility therefore received high economic weights (i.e LG, SC, SR and TN). Traits with low genetic variance had less power in distinguishing the cold tolerance and susceptible genotypes and received low economic weight (i.e SH). Based on the index (the sum value of the five traits), SCRID091-20-2-2-4-4 was ranked first followed by MET P27. The lowest value was obtained for ARS126-3-B-1-2 (11). The highest index value for SCRID091-20-2-2-4-4 showed that this genotype performed relatively better for the five collected traits and revealed its tolerance to cold stress. The lower index value of ARS126-3-B-1-2 (11) genotype pointed to the susceptibility of the genotype to cold stress. Base index selection was able to identify and group the cold tolerant and susceptible rice genotypes in this screening condition which fitted well with visual selection and tolerance indices selection.

Tolerance indices tell us the amount of yield reduction due to cold stress as compared to non-stress conditions. The cold stress treatment used in this study caused up to 100% yield reduction for highly susceptible genotypes. Low Stress Susceptibility Index (SSI) value implied tolerance of the genotypes under applied stress. SCRID091-20-2-2-4-4 followed by MET P20 had low SSI value. Numerous studies confirmed that the lower the SSI value, the more tolerant the genotype is and it was reported as a good index to identify stress tolerance and susceptible genotypes

(Guttieri *et al.*, 2001; Talebi *et al.* (2009) ; Shiranirad and Abbasian, 2011; Zdravković *et al.*, 2013). High Tolerance index (TOL) value indicated high loss due to the applied stress and susceptibility of the genotypes. The lower the TOL value, the more desirable the genotypes (Rosielle and Hamblin, 1981). Based on this approach the desired genotypes in this study was SCRID091-20-2-2-4-4 which had low TOL value, followed by MET P20 and the undesirable genotype was ARC39-145-P-2 (5). The higher yield under stress to non-stress ration (YSI) suggested tolerance of genotypes (Bousslama and Schapaugh, 1984). In all values of tolerance indices, SCRID091-20-2-2-4-4 ranked first followed by MET P20 while the worst genotype was ARS126-3-B-1-2 (11) which ranked last for tolerance indices. According to this study, yield reduction rate, TOL, SSI and YSI were the best fitted tolerance indices to identify cold tolerant genotypes. A similar study reported that these indices were the more powerful stress discriminators in severe cold stress condition (Talebi *et al.*, 2009). In general, tolerance indices were able to distinguish genotypes which tolerate cold stress and showed small performance difference as compared to non-stressed treatment. This revealed that genotypes with low TOL, SSI and YSI can give better yield in presence of cold stress. In other words, farmers may face lower yield penalty if they grew these varieties with low TOL, SSI, and YSI.

3.5 Conclusions and Recommendation

This study identified 8 highly cold tolerant, 15 (tolerance), 9 (moderately tolerant), 13 (susceptible) and 5 (highly susceptible) rice genotypes from 50 rice genotypes tested at seedling stage. MET P27, GIZA 177, MET P23, SCRID091-20-2-2-4-4, NERICA 1, MET P5, MET P20 and MET P60 were the highly cold tolerant rice genotypes selected for all applied selection methods. Apart from these (GIZA 177, SCRID091-20-2-2-4-4 and NERICA 1), the other identified cold tolerant genotypes had long grain size. GIZA 177 (short grain), SCRID091-20-2-2-4-4 (medium grain size) and NERICA 1 were also identified as cold tolerance in the other study. These short and medium grain size rice could be used as donors in breeding for cold tolerance.

Abbreviations

Df.	Degree of freedom
GRiSP	Global Rice Science Partnership

IRRI	International Rice Research Institute
LSD	Least Significance Difference
MUARIK	Makerere University Agricultural Research Institute Kabanyolo
NaCRRI	National Crop Resources Research Institute
NEWEST	Nitrogen- use Efficient, Water-use Efficient and Salt Tolerance
ReML	Restricted Maximum Likelihood
SES	Standard Evaluation System
SSI	Stress Susceptibility Index
STI	Stress Tolerance Index
TOL	Tolerance Index
YP	Yield under non-stress
YS	Yield under stress

Declaration

Ethical approval and consent to participate

No applicable

Consent for publication

No applicable

Availability of supporting data

All data generated and analyzed in this study included in the figures and tables presented in the manuscript

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No competing interest

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Authors' contributions

Kidist Tolosa was designed research and performed research and wrote the manuscript, Richard Edema, Jimmy Lamo supervised research, Nigat Tilahun, Desta Abebe and Worku Kebede help in analyzed and edited the manuscript. The author(s) read and approved the final manuscript.

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APPENDICES

Appendix 1: Means performance of 50 rice genotypes screened at seedling stage (at 10 °C for 10 days) and their comparison mean in non-stress condition

Line name	Leaf growth	Seedling Color	Seedling height(cm)		Reduction rate (%)	Tiller number		Reduction rate (%)	Survival rate	Grain yield (g)/ pot	
	Stressed	Stressed	Stressed	N-stressed		Stressed	N-stressed		Stressed	Stressed	N-stressed
MET 10	6.3	5.8	25.8	44.8	42.4	1.6	3.2	49.6	54.2	32.8	82.6
MET 27	1.0	1.1	33.4	38.9	14.2	2.9	3.3	12.7	100.0	49.4	53.7
MET 9	2.6	2.5	34.1	45.1	24.6	2.4	3.0	20.7	100.0	66.3	81.4
ARS126-3-B-1-2 (11)	9.0	9.0	0.1	30.9	99.8	0.0	3.7	100.1	0.0	0.0	71.4
MET 5	1.3	1.5	29.0	38.1	23.9	2.3	3.1	27.1	100.0	73.0	79.6
MET 21	6.6	6.7	25.1	37.6	33.1	1.3	2.3	41.3	58.3	45.8	85.7
MET 19	4.3	6.1	26.0	38.4	32.3	2.0	2.7	26.4	58.3	51.8	74.7
MET 12	6.2	6.2	26.4	39.9	33.8	1.4	3.3	57.4	58.3	49.4	90.1
MET 37	2.5	2.7	29.7	42.7	30.5	1.9	3.0	36.4	95.8	53.9	64.1
MET 38	6.5	5.5	25.8	40.9	37.1	1.8	3.0	42.0	58.3	34.5	83.4
MET3	2.1	2.5	26.1	37.3	30.0	1.6	2.2	27.6	100.0	57.6	66.5
ARC36-4-ET-2 (3)	8.7	8.7	14.7	32.0	54.2	0.6	3.7	83.8	29.2	11.6	63.1
MET 1	5.5	5.6	25.1	39.0	35.8	1.3	2.6	50.4	54.2	43.2	73.3
MET 30	6.0	5.1	23.1	32.4	28.6	1.4	3.1	56.1	70.8	38.6	68.3
MET29	3.8	5.9	25.6	39.3	35.0	1.8	2.5	26.5	50.0	57.4	80.9
MET 2	1.8	1.9	25.6	38.8	34.1	2.3	2.9	18.1	100.0	63.4	74.2
MET 32	1.7	1.7	29.9	43.4	31.1	1.7	2.6	32.6	100.0	63.7	74.9
MET 26	6.4	5.6	25.1	40.2	37.6	1.7	2.8	41.4	62.5	50.8	86.7
MET 11	1.8	1.8	26.8	41.4	35.2	2.1	3.1	32.2	100.0	72.9	88.6
MET 6	5.9	5.5	26.0	39.7	34.5	1.2	2.4	50.3	75.0	44.3	67.5
MET 14	6.5	6.5	25.1	36.9	31.9	1.9	2.4	20.0	62.5	34.4	73.6
ARC39-145-P-2 (5)	9.0	9.1	0.1	26.7	99.6	1.0	5.3	81.1	0.0	0.0	76.3
MET 40	2.6	2.6	32.5	42.1	22.8	2.2	2.7	15.7	100.0	49.7	56.4
MET 20	1.2	1.4	27.6	35.7	22.8	2.2	2.6	14.4	100.0	63.1	68.6
MET28	4.1	5.1	28.9	43.2	33.0	2.0	2.6	20.6	62.5	56.2	76.4
ARC36-2-P-2-54 (2)	5.7	6.9	16.9	24.9	31.9	1.4	2.6	47.2	29.2	27.2	62.5
MET 33	6.2	4.5	26.2	35.2	25.6	1.7	2.1	21.8	79.2	35.2	66.9

Line name	Leaf growth	Seedling Color	Seedling height(cm)		Reduction rate (%)	Tiller number		Reduction rate (%)	Survival rate	Grain yield (g)/ pot	
	Stressed	Stressed	Stressed	N-stressed		Stressed	N-stressed		Stressed	Stressed	N-stressed
MET 36	3.1	1.8	26.1	41.3	36.8	2.0	3.7	45.2	100.0	68.4	85.6
NERICA 1	1.3	2.2	29.1	35.5	18.0	2.7	3.3	17.6	100.0	85.9	97.0
MET 17	1.8	2.2	27.0	38.3	29.6	2.0	2.6	25.3	100.0	65.0	79.1
MET 7	5.8	5.8	26.4	45.0	41.4	1.3	3.2	58.6	62.5	38.4	66.5
MET 13	5.7	6.2	22.3	38.0	41.4	1.7	2.7	35.2	58.3	46.4	87.2
MET 22	3.0	2.7	26.6	38.9	31.5	2.9	3.2	7.6	100.0	54.9	69.7
WITA 9	7.5	6.9	17.4	32.9	47.3	1.8	5.3	66.8	33.3	27.9	63.2
MET 60	1.3	1.7	33.1	39.6	16.4	3.0	3.3	10.8	100.0	61.7	69.4
MET 39	2.9	2.8	23.8	36.5	34.8	2.1	3.0	29.7	100.0	46.1	57.8
ARC36-2-1-2	7.7	8.3	8.5	33.6	74.7	0.5	4.1	87.6	12.5	30.2	83.0
MET 31	2.9	3.0	28.6	41.6	31.2	2.6	3.5	24.0	79.2	61.5	77.6
MET 8	3.6	4.3	22.8	38.2	40.3	2.4	3.9	37.4	79.2	46.7	90.7
MET 25	4.8	3.8	28.4	39.5	28.1	2.0	2.9	31.9	62.5	48.9	75.1
MET 35	3.9	4.1	25.0	40.3	38.0	1.4	3.1	53.8	70.8	56.8	85.4
MET 24	2.1	2.2	26.0	39.8	34.6	2.0	2.9	31.9	100.0	56.6	73.5
MET23	1.1	1.0	32.1	37.1	13.7	2.2	2.9	24.0	100.0	69.0	79.0
MET34	3.2	3.4	24.5	35.6	31.2	2.1	3.4	37.6	60.0	55.9	75.2
MET 18	2.5	3.4	25.2	40.3	37.4	2.3	4.6	50.8	95.9	76.3	88.1
MET 16	2.2	3.6	28.0	42.6	34.1	1.7	2.5	34.7	100.0	49.7	61.6
GIZA 177	1.0	0.7	30.2	38.0	20.5	2.8	4.3	35.3	100.0	64.8	72.0
MET 15	4.1	6.1	27.0	39.4	31.5	2.2	2.9	24.0	62.5	50.2	76.9
SCRID091-20-2-2-4-4	1.2	1.4	37.2	45.0	17.3	2.6	3.3	20.3	100.0	71.8	77.5
MET 4	3.5	3.9	23.6	38.2	38.2	1.5	3.2	52.1	79.2	38.8	58.8
Mean	4.0	4.2	23.1	38.3		2.0	74.3		3.5	52.0	74.8
LSD (5%)	0.9	1.2	4.6	7.5		0.8	ns		16.0	29.1	87.9

Appendix 2: Tolerance indices of 50 rice genotypes under stress and non-stress condition for grain yield per pot

Line name	GY/pot (stressed)	Gy/pot (Non-stressed)	% Reduction	SSI	TOI	YSI
SCRID091-20-2-2-4-4	71.8	77.5	7.3	0.2	5.7	0.9
MET P20	63.1	68.6	8.0	0.3	5.5	0.9
MET P27	49.4	53.7	8.1	0.3	4.4	0.9
MET P5	73.0	79.6	8.2	0.3	6.5	0.9
GIZA 177	64.8	72.0	9.9	0.3	7.2	0.9
MET P60	61.8	69.4	11.0	0.4	7.6	0.9
NERICA 1	85.9	97.1	11.5	0.4	11.2	0.9
MET P40	49.7	56.4	12.0	0.4	6.7	0.9
MET P23	69.0	79.0	12.7	0.4	10.0	0.9
MET P3	57.6	66.5	13.3	0.4	8.8	0.9
MET P18	76.3	88.1	13.4	0.4	11.8	0.9
MET P2	63.4	74.2	14.5	0.5	10.8	0.9
MET P32	63.7	74.9	15.0	0.5	11.2	0.9
MET P37	53.9	64.1	15.9	0.5	10.2	0.8
MET P11	72.9	88.6	17.7	0.6	15.7	0.8
MET P17	65.0	79.1	17.8	0.6	14.1	0.8
MET P9	66.3	81.4	18.5	0.6	15.1	0.8
MET P16	49.8	61.6	19.2	0.6	11.9	0.8
MET P36	68.4	85.6	20.1	0.7	17.2	0.8
MET P39	46.1	57.8	20.3	0.7	11.7	0.8
MET P31	61.5	77.6	20.7	0.7	16.1	0.8
MET P22	54.9	69.7	21.2	0.7	14.8	0.8
MET P24	56.6	73.5	22.9	0.8	16.9	0.8
MET P34	55.9	75.2	25.7	0.8	19.3	0.7
MET P28	56.2	76.4	26.4	0.9	20.2	0.7
MET P29	57.4	80.9	29.1	1.0	23.5	0.7
MET P19	51.8	74.7	30.6	1.0	22.8	0.7
MET P35	56.8	85.4	33.5	1.1	28.6	0.7
MET P4	38.8	58.8	34.1	1.1	20.1	0.7
MET P6	44.3	67.5	34.3	1.1	23.2	0.7
MET P15	50.2	76.9	34.8	1.1	26.8	0.7
MET P25	48.9	75.1	34.9	1.2	26.2	0.7
MET P1	43.2	73.3	41.1	1.4	30.1	0.6
MET P26	50.8	86.7	41.4	1.4	35.9	0.6
MET P7	38.4	66.5	42.3	1.4	28.1	0.6
MET P30	38.7	68.3	43.4	1.4	29.7	0.6
MET P12	49.4	90.1	45.2	1.5	40.7	0.6
MET P21	45.8	85.7	46.6	1.5	39.9	0.5
MET P13	46.4	87.2	46.7	1.5	40.7	0.5

MET P33	35.2	66.9	47.4	1.6	31.7	0.5
MET P8	46.7	90.7	48.5	1.6	44.0	0.5
MET P14	34.4	73.6	53.2	1.8	39.2	0.5
WITA 9	28.0	63.2	55.8	1.8	35.2	0.4
ARC36-2-P-2-54 (2)	27.2	62.5	56.5	1.9	35.3	0.4
MET P38	34.5	83.4	58.6	1.9	48.9	0.4
MET P10	32.8	82.6	60.3	2.0	49.8	0.4
ARC36-2-1-2	30.2	83.0	63.7	2.1	52.8	0.4
ARC36-4-ET-2 (3)	11.6	63.1	81.7	2.7	51.6	0.2
ARS126-3-B-1-2 (11)	0.0	71.4	100.0	3.3	71.4	0.0
ARC39-145-P-2 (5)	0.0	76.3	100.0	3.3	76.3	0.0
LSD (5%)	29.1	32.8				
Mean	50.0	74.8	33.1	1.1	24.9	0.7
Min	0.0	53.7	7.3	0.2	4.4	0.0
Max	85.9	97.1	100.0	3.3	76.3	0.9