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Research paper

Estimating the genotype by environment interaction among diverse rice genotypes identified under artificial conditions

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The aim of this study was to estimate the genotype by environment interaction of artificially identified cold tolerant rice genotypes in the highland cooler parts of Uganda. The best twenty three cold tolerant genotypes and two susceptible genotypes were evaluated in seven environments, which were characterized by cold weather. Five by five alpha lattice design with three replications was used in each environment. Analysis of variance revealed significant GEI effect in most of the cold tolerance indicator traits. Leaf wilting score was one of the major measures of cold stress observed on evaluated genotypes and it was chosen for demonstration of AMMI ANOVA and GGE-biplot. AMMI analysis showed there were highly significant environments, genotypes and GEI effects on leaf wilting score. The partitioning of total sum of squares (TSS) revealed that the environment effect was a predominant source of variation followed by GEI and genotype. The first two PCA on GGE-biplot explain 72.72% of the total variation of GEI. In general, rice genotypes SCRID091-20-2-2-4-4, MET P9, MET P40, NERICA 1 and GIZA 177 took short duration to mature, had better yield and were cold tolerant in most of test environments.

Key words: *Genotype by environment interaction (GEI), rice crop, cold stress*

INTRODUCTION

Rice is one of the major food crops and it is the second most important cereal crop for Uganda after Maize (Akongo et al., 2016). The crop is also one of the strategic crops for alleviating poverty in the country, especially for rural poor communities (Odogola, 2006) and hence become a major cash crop in many districts of Uganda (MAFAP, 2012). However, since 2000, the demand for rice in Uganda has grown at an average rate of about 9.5 percent per year (Ahmed, 2012) highlighting the need to expand the area under rice production and develop rice varieties which resist and/or tolerate the adverse effect of different environments. Due to lack of improved rice cultivars which are tolerant to the cold stress in highlands of Uganda, rice production is restricted to warmer low land areas. However, there is an opportunity of enough rainfall and land resource in highland areas of the country with the willingness of farmer to grow rice (Haneishi, 2014). This opportunity could hence play a crucial role in area intensification and increment of rice productivity. In a previous study done to identify cold tolerance rice, (Nyiramugisha et al., 2017) identified short-sized grain genotypes with promising cold tolerance trait. However, these genotypes were not the preferred type for

Ugandan consumers. In an effort to identify cold tolerant rice genotypes, the current study screened mostly long speed grain genotypes. The best 23 and two susceptible genotypes were selected. Arable areas in the highlands of Uganda are also variable, ranging from about 1700 masl to above 2500 masl. It is therefore important to determine the extent to which cold tolerant rice can grow in this diverse environment for further evaluation in the highland areas of Uganda. Therefore, the objective of this study was to estimate the genotype by environment interaction in the highland parts of Uganda. Prior to this study, no other studies had evaluated rice in highland areas of the country.

MATERIALS AND METHODS

Study Area

This research was conducted in seven environments which are characterized by cold weather. The experiment was conducted in 2017B and 2018A growing season. The test environments were: KAZARDI-Kabale (2017B and 2018A), BZARDI-Mbale (2017B), Zombo (2017B and 2018A), Bushenyi (2018A) and Buligeni (2018A). These environments are located in southwest, Eastern, northwest, western and north east parts of Uganda, respectively, and are approximately 420km, 245km, 382km, 365km and 221km far from the capital city of the country (Kampala), respectively (https://en.wikipedia.org, 2018). Monthly minimum and maximum temperatures from planting to the last growth stage of the experiment were obtained from the metrological stations, (Https://www.accuweather, 2017-2018.) and (https://www.worldweatheronline.com/, 2018-2018). The details of monthly minimum and maximum temperatures, rain fall and humidity of all environments are presented in (Appendix 14) and general agro- ecology of test environments in Table 1.

Experimental materials

A total of 23 selected cold tolerant rice genotypes and two susceptible genotypes were evaluated in seven environments for estimation of genotype by environment interaction. Of the 23 cold tolerant rice genotypes 21 were long grain size. The details about the genotypes (pedigree, source and grain type information) are presented in Table 2 below.

Experimental design

A total of 25 rice genotypes were evaluated in seven environments using alpha lattice design (5 blocks by 5 genotypes per block) with three replications. Forty hills were established on a 1.2 m x 2.0 m plot size, with a spacing of 20 cm between plants and 30 cm between rows (NERICA promotion project, 2011). The spacing between blocks and replications was 0.5 m and 1.0 m, respectively. Four seeds were planted per hill through dibbing planting method, and were thinned 21 days after germination. Di-ammonium Phosphate (DAP) fertilizer with (18% phosphorous) was applied at planting at rate of 100 kg per hectare. Urea fertilizer (46% Nitrogen) was applied at a rate of 100 kg per hectare in two rounds: 21days after planting and at the crop's booting stage.

Data collection

The data were collected on ten representative plants from two central rows of each plot for each of the traits listed in Table 3. Visual data namely, leaf growth/ leaf wilting score, seedling color/yellowing score and seedling vigor score were taken based on standard evaluation system (SES) developed by IRRI (2013). Leaf growth, seedling color, survival rate, seedling vigor, seedling height and tiller number were recorded at 38 days after planting in a similar way to controlled cold screening in cold growth chamber (Section 3.2.4). Yield data were obtained from three environments (Zombo 2017B (E1), Zombo 2018A (E4) and Bulegeni 2017B (E7) due to very low temperatures experienced in (Kabale 2017B (E2), Mbale 2017B (E3) and Kabale 2018A (E5) which prolonged heading and highly affected grain filling and drought conditions during the cropping season in Bushenyi 2018A (E6).

Statistical analysis

The collected data were subjected to analysis of variance using linear mixed model (ReLM) procedure (Genestat software, 18th edition) (Payne et al., 2015), in which genotypes were considered to be a fixed term whereas the replication and block were taken as random terms. On the other hand, in the

Table 1. General agro-ecology (maximum and minimum temperatures, altitude, latitude and longitude) of test environments

	Agro- ecology of test environment				
	Temperature (°C)		Geographical coordinates		
Environment	[8]Min	[9]Max	Altitude [10](masl)	Latitude	Longitude
[1]E1	13.3	28.8	1609	2.3°N	31°E
[2]E2	10.0	22.0	2066	1°15' S	29°56'60E
[3]E3	12.9	26.1	1886	1.03° N	34.2° E
[4]E4	13.3	28.8	1609	2.3°N	31°E
[5]E5	10.0	22.0	2066	1°15'S	29°56'6E
[6]E6	15.6	24.5	1607	0°32′13″ S	30°11′08″ E
[7]E7	16.0	24.0	1600	1°4'29N	34°10'39E

[1]Zombo2017B, [2]KAZARDI 2017B-Kabale, [3]BZARDI 2017B-Mbale, [4]Zombo 2018A, [5]KAZARDI 2018A-Kabale, [6]Bushenyi 2018A, [7]Bulegeni 2018A;, [8]Minimum, [9]Maximum, [10]meters above sea level KZARDI= Kachwekano Zonal Agricultural Research Development Institute, BZARDI=Buginyanya Zonal Agricultural Research Institute

Source: Buginyanya metrological station, Kabale metrological station, www.accuweather.com and www.worldweatheronline.com

Table 2. Characteristics (pedigree, source and grain type) of rice genotypes selected for evaluation during the 2017B and 2018A growing seasons

Code	Line Name	Pedigree	Other information	Source	Grain type
G1	SCRID091-20-2-2-4-4	SCRID091-20-2-2-4-4	Cold tolerant	Tanzania	Medium
G2	MET P27	ART27-190-1-3-3-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G3	GIZA 177	GIZA 177	Japonica subs pecieses	Egypt	Short grain
G4	MET P32	ART27-190-7-3-2-4-3-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G5	MET P23	ART27-58-8-1-1-4	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G6	MET P11	ART34-86-2-1-B-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G7	MET P16	ART35-272-1-2-B-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G8	MET P60	PCT-4\0\0\1>295-2-3-1-3-3-M	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G9	MET P17	ART27-58-7-1-2-2-2-2	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G10	MET P40	ART27-190-1-4-2-1-1-3	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G11	NERICA 1	NERICA 1	Cold tolerant	Africa Rice	Long grain
G12	MET P22	ART27-58-3-2-1-1	*O.barthi* interspecific lines	Africa Rice	Long grain
G13	MET P20	ART27-58-7-2-2-3	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G14	MET P31	ART15-7-16-38-1-B-B-2	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G15	MET P3	ART35-114-1-6N-2	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G16	MET P24	ART3-7L9P8-3-B-B-2-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G17	MET P9	ART35-4-1-5D-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G18	MET P39	ART27-190-7-6-4-2	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G19	TOX 3058-28-1-1-1	WITA 9	Cold susceptible	IR 2042-178-1/CT19	Long grain
G20	ARC36-2-1-2	ARC36-2-1-2	Cold susceptible	Africa Rice-Benin,	Long grain
G21	MET P2	ART34-82-1-7N-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G22	MET P18	ART27-58-3-2-1-4	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G23	MET P37	ART27-122-19-3-1-3	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G24	MET P36	ART27-122-19-3-1-2-1-1	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain
G25	MET P5	ART34-79-1-2N-2	*O.barthi* interspecific lines	Africa Rice-Ibadan, Nigeria	Long grain

combined analysis, the genotypes were treated as a fixed term and environments and genotype by environment interaction as random terms. The analysis of variance was done for each trait in each environment. The linear model in each environment was:

$Y_{ijk} = \overline{\overline{Y}} + G_i + R_j + B_{kj} + e_{ijk}$

Where, Y_{ijk} = The observed value of trait for the i^th^ genotype in the k^th^ block nested within the j^th^ replicate, $\overline{\overline{Y}}$ = grand mean, G_i = the effect of the i^th^ genotype, R_j =effect of j^th^ replicate, $B_{k(j)}$ is the effect of the k^th^ block nested within the j^th^ replicate and e_{ijk} = random residual or error term.

Table 3. Descriptions and list of traits collected for genotype by environment interaction study

Traits	Acronym	Descriptions
Leaf growth	LG	The cold damage on leaf morphology/leaf wilting scales of 1-9, which developed by IRRI (1-3: tolerant , 4-5: intermediate and 7-9: susceptible)
Seedling color	SC	The damage of cold on the color of leaf on a 1-9 scale developed by IRRI; where 1: dark green, 3: light green, 5: yellow, 7: reddish brown and 9: dead.
Survival rate	SR	Percentage of seedling that survive (number of survived seedlings / number of germinated seedlings x 100)
Seedling vigor	SV	Scale 1-9 developed by IRRI 1: extra vigorous (very fast growing; plants at 5-6 leaf stage have 2 or more tillers in most plant population), 3: vigorous (fast growing; plants at 4-5 stage have 1-2 tillers in majority of population, 5: normal (plant at 4 leaf stage) 7: Weak (plants somehow stunted 3-4 leafs) 9: very weak (stunted growth and yellowing of leaves)
Seedling height	SH	The height of seedling from the base of the shoot to the tallest leaf
Vegetative stage tiller number	TN	Number of tillers 38 days after planting
Days to flowering/Heading	DTH	Number of days from planting to 50% of the plot headed
Panicle Exsertion	Pan EX	The extent by which the panicle exerted from flag leaf, 1: enclosed (panicle partially or entirely enclosed with flag leaf), 3:Partly exserted (panicle base is slightly beneath the collar of the flag leaf), 5: Just exserted (panicle base coincides with the collar of the flag leaf), 7: Moderately well exserted (panicle base is above the collar of the flag leaf), 9: Well exserted (panicle base appears well above the collar of the flag leaf blade) taken at maturity from ten randomly selected inner row plants
Flag leaf length	FL	The length the topmost leaf from collar to the tip of leaf blade measured from ten randomly selected plants in the inner row
Flag leaf width	FW	Enter actual measurements, in centimeters of the widest portion of the leaf blade just below the flag leaf at the time of heading
Plant height	PH	The height of ten inner row plants measured from the soil surface to the tip of the tallest panicle measured at physiological maturity stage.
Total number of tiller at reproductive stage	TTN	The number of branches arising from the main plant and counted close to maturity stage.
Days to maturity	DTM	Number of days from planting to 85% physiological maturity
Grain yield	GY	The yield of the two central rows (0.3m*2m) at physiological maturity
Grain filling ration	GFR (%)	The ration of filled grain to total yield (filled plus unfilled)

Source: Standard evaluation system International Rice Research Institute (IRRI), 2013

The analysis of variance across environments was based on the statistical model below:

$$Y_{ijkl}=\bar{\bar{Y}} + G_i + E_j + \mathrm{GE}_{ij} + \mathrm{R/E}_{kj} + \mathrm{B/(R,E)}_{lkj} + \mathrm{E}_{ijkl}$$

Where, Y_{ijkl} = observed value across environments, $\bar{\bar{Y}}$= grand mean,G_i, E_j, GE_{ij} were the genotype, the environment and the genotype by environment interaction effect, respectively, $\mathrm{R/E}_{kj}$ = kth replication effect of the nested within the jth environments, $\mathrm{B/(R,E)}_{lkj}$ = effect of the lth block nested within the kth replication and jth environments and e_{ijkl} =the experimental error.

Leaf growth/leaf wilting score was also analyzed using genotype plus genotype by environment (GGE) bi-plot methodology, to visualize the genotype by environment interaction (GEI) pattern (Yan & Holland, 2009). The scores were transformed using inverse function plus one. Pearson's correlation analysis was performed for seedling traits under cold growth chamber and field condition.

RESULTS AND DISCUSSION

The study demonstrated the existence of GEI among rice genotypes evaluated in the seven environments. The significant GEI observed among the genotypes evaluated proved that genotypes responded differently to the variable environmental conditions. High significance variation was observed among rice genotypes for most of the cold stress parameters namely, leaf growth/leaf wilting score, seedling color/leaf yellowing score, survival rate, seedling height, days to heading, panicle exsertion, plant height, flag leaf length, flag leaf width, total tiller number, days to maturity, grain yield and grain filling ration collected at different growth stages of plants. Mean squares of the cold tolerance cold tolerant traits measured at the seedling stage are presented in Table 4. Results from the analysis showed significant differences ($p<0.05$, $p<0.01$ and $p<0.001$) among genotypes evaluated for most of the traits, indicating presence of genetic diversity

Table 4. Mean squares for seedling traits measured in seven environments during the 2017B and 2018A growing seasons

	Mean square							
Traits	**Environment**	**REP**	**Rep/block**	**Genotypes**	**Residual**	**LEE**	**SED**	**CV%**
		Df=2	**Df=12**	**Df=24**	**Df=36-48**	**Df=23-48**		
Leaf growth	^{1}E1	0.6^{ns}	2.2^{*}	3.4^{*}	1.3	1.5	1.0	35.2
	^{2}E2	1.9^{ns}	1.6^{ns}	7.1^{***}	1.0	0.7	0.9	27.1
	^{3}E3	4.6^{ns}	2.5^{ns}	6.1^{***}	1.3	1.5	1.0	33.8
	^{4}E4	1.9^{ns}	-	8.5^{***}	0.7	-	0.7	50.5
	^{5}E5	3.6^{ns}	1.6^{ns}	5.7^{***}	0.9	1.0	0.8	34.5
	^{6}E6	0.3^{ns}	0.8^{ns}	2.9^{**}	0.7	0.7	0.7	36.4
	^{7}E7	2.1^{ns}	1.1^{ns}	1.9^{*}	0.8	0.9	0.6	58
Seedling color	^{1}E1	0.5^{ns}	-	2.9^{ns}	1.8	-	1.1	48.3
	^{2}E2	0.7^{ns}	-	7.5^{***}	1.5	-	1.0	33.3
	^{3}E3	2.6^{ns}	3.4^{ns}	6.7^{***}	1.7	2.0	1.1	49.9
	^{4}E4	1.5^{**}	1.0^{ns}	6.7^{ns}	0.6	0.6	0.6	35.7
	^{5}E5	2.4^{ns}	-	5.9^{**}	1.2	-	1.0	34.9
	^{6}E6	0.76^{ns}	-	2.7^{**}	1	-	0.8	43.3
	^{7}E7	1.9^{*}	-	4.5^{**}	0.6	-	0.6	55.9
Survival rate (%)	^{1}E1	939^{ns}	263.64^{**}	550.6^{***}	69.94	92.2	7.8	16.4
	^{2}E2	177.3^{ns}	-	582.9^{***}	149.5	-	7.7	14.4
	^{3}E3	3969.3^{ns}	991.4^{***}	900.1^{**}	254.8	338.4	15.0	43.7
	^{4}E4	153.8^{ns}	90.4^{ns}	424.5^{***}	90	90.1	7.8	13.3
	^{5}E5	131.98^{ns}	-	507.4^{***}	58.31	-	6.2	10.7
	^{6}E6	69.7^{ns}	61.8^{ns}	450.3^{***}	68.8	67.3	6.7	16
	^{7}E7	60.1^{ns}	42.3^{ns}	207.2^{***}	27.1	29.7	4.5	7.7
Seedling vigor	^{1}E1	11.3^{**}	2.1^{**}	4.0^{***}	0.6	0.8	0.7	25.3
	^{2}E2	0.7^{ns}	2.3^{ns}	9.7^{***}	1.6	1.7	1.1	33.3
	^{3}E3	1.1^{ns}	1.3^{ns}	5.6^{**}	1.1	1.2	0.9	25.7
	^{4}E4	1.6^{ns}	-	11.8^{**}	1.0	-	0.8	51.1
	^{5}E5	12.9^{ns}	-	6.8^{*}	1.5	-	1.0	30.3
	^{6}E6	13.8^{**}	2.3^{ns}	5.9^{***}	1.7	1.8	1.1	36.5
	^{7}E7	0.7ns	-	4.3^{**}	1.7	-	1.1	33.8
Seedling height	^{1}E1	214.5^{**}	88.7^{**}	231.5^{***}	21.9	26.6	4.9	15.5
	^{2}E2	3.14^{ns}		19.1^{*}	10.6		2.7	15.5
	^{3}E3	166.0^{ns}	56.3^{*}	46.8^{***}	13.8	13.9	3	15.5
	^{4}E4	1678.2^{***}	81.7^{ns}	205.8^{***}	55.3	95.3	8	17.1
	^{5}E5	43.1^{ns}	-	108.8^{***}	26.3	-	4.2	15.4
	^{6}E6	87.2^{ns}	84.3^{ns}	193.2^{**}	80.4	13.9	7.4	21.1
	^{7}E7	145.3^{ns}	145.5^{ns}	155.3^{ns}	104.2	111.7	8.6	23.1
Tillers number	^{1}E1	1.5^{**}	0.2^{ns}	0.1^{ns}	0.1	0.1	0.3	13.6
	^{2}E2	0.02^{ns}	-	1.68^{***}	0.3	-	0.4	16.3
	^{3}E3	2.6^{**}	-	0.7^{***}	0.3	-	0.4	17.2
	^{4}E4	697.2^{**}	101.1^{ns}	0.9^{ns}	168.5	2.0	1.0	20.9
	^{5}E5	1.7^{ns}	0.5^{ns}	0.4^{ns}	0.2	0.3	0.4	19.6
	^{6}E6	0.02^{ns}	-	0.43^{ns}	0.3	-	0.5	12.3
	^{7}E7	13.7^{ns}	1.0^{ns}	1.0^{***}	0.8	0.8	0.7	16.0

ns= non-significant, $^{*, **,}$ and***significant at probability level of 0.05, 0.01 and 0.001 respectively, ^{1}Zombo2017B, ^{2}KAZARDI 2017B-Kabale, ^{3}BZARDI 2017B-Mbale, ^{4}Zombo 2018A, ^{5}KAZARDI 2018A-Kabale, ^{6}Bushenyi 2018A, ^{7}Buligeni 2018A; Df= Degree of freedom, Rep=Replication, Rep/block= Block nested in replication, LEE= Lattice effective error, SED= Standard error of difference, CV= Coefficient of variation among these genotypes.

Performance of selected rice genotypes across environments for seedling stage traits

(i). Leaf growth/leaf wilting score

The evaluated 25 rice genotypes were categorized into five based on cold tolerance levels on their leaf growth/leaf wilting score in the different environments. The score ranged between 0 to 1 for highly tolerant, 1.5 to 3.49 for tolerant, 3.5 to 5.49 moderately tolerant, 5.5 to 7.4 for susceptible genotypes and 7.5 to 9 for highly susceptible. Results for leaf growth/leaf wilting score are presented in Table 5 and Figure 1. In environment E1, 12 tolerant, 11 moderately tolerant and 2 susceptible rice genotypes were identified. In environments E2, 8 tolerant, 10 moderately tolerant and 7 susceptible genotypes; in E3: 12 tolerant, 11 moderately tolerant and 2 susceptible rice genotypes; in E4: 8 highly tolerant, 12 tolerant, 3 moderately tolerant and 2 susceptible genotypes; in E5: 2 highly tolerant, 12 tolerant, 9 moderately tolerant and 2 susceptible genotypes; in E6: 5 highly tolerant, 17 tolerant and 3 moderately tolerant and in E7: 7 highly tolerant, 13 tolerant and 1 susceptible genotypes were identified. None of the genotypes were highly tolerant in E1, E3 and E4.

More number (8 genotypes) of highly tolerant genotypes were identified in E4 followed by E7 (7 genotypes) and E6 (5 genotypes) (Figure 1). In general, 48% to 62% of the evaluated genotypes were categorized under cold tolerant in all test environments except for E2, in which most of the genotypes were grouped under moderately tolerant for leaf wilting traits scored at seedling stage (Figure 1). Mean leaf growth/leaf wilting score results (Table 5) showed that genotypes G13, G1, G8, G24, G10 and G2 were the best tolerant genotypes in E1, E2, E3, E4, E5, E6 and E7 respectively with mean scores between 1.0 and 1.9. Genotypes G19 and G20 were the worst performing genotypes in all environments, with mean scores of between 4.4 and 8.2 (Table 5). Visual assessment on morphology character is a common practice in evaluation of cold tolerance rice at seedling stage. Primary visual symptoms such as wilting and leaf yellowing are highly correlated with cold stress and they are the physiological symptoms observed after the cold stress (Su *et al*., 2010; Yang et al., 2012). It has been reported that low temperature reduces hydraulic conductivity of rice roots leading to dehydration and consequently wilting of the plant, even if there is water in the soil (Murai Hatano et al., 2008). Cold stress induced dehydration also causes damage of plasma membrane, which is composed of proteins and lipids. Plasma membrane is composed of proteins and lipids. The lipids components of plasma membrane are composed of saturated and unsaturated fatty acid (Sudesh, 2010). In cold tolerant plants, the proportion of unsaturated fatty acids is more than that of saturated fatty acids resulting in low temperature transition. On the other hand, cold sensitive genotypes had high proportion of saturated fatty acids than unsaturated fatty acids resulting in high transition temperature (Sudesh, 2010). IRRI (2013) developed a standard evaluation system (SES) scale to distinguish tolerant and susceptible rice genotypes, which has been used by several studies for cold tolerance evaluation (Andaya and Mackill, 2003; Kim and Tai, 2011; Hyun et al., 2016). Leaf growth/leaf wilting score is one of an essential measurement for cold stress damage. The result of the current study showed that rice genotypes had significantly different leaf growth/ leaf wilting scores in all test environments, suggesting their variable response in different environmental conditions. Although low minimum temperatures were observed during the cropping season, some rice genotypes performed better and exhibited low leaf

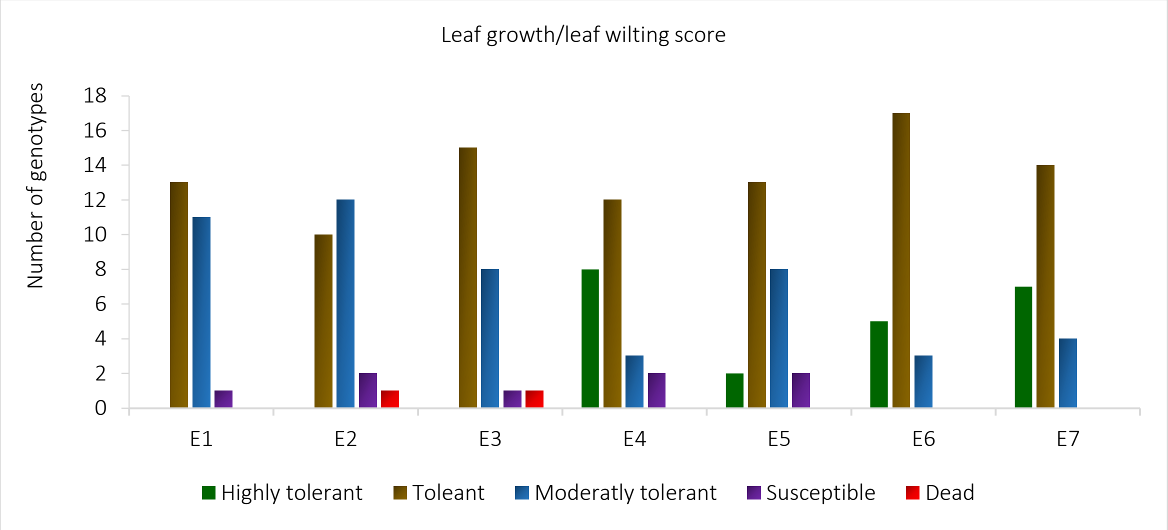

Figure 1. Frequency distribution of selected rice genotypes for leaf wilting score in seven evaluated at seeding stage during the 2017B and 2018A growing seasons

Where: E1=Zombo2017B, E2=KAZARDI-kabale 2017B, E3=BZARDI-Mbale 2017B, E4=Zombo 2018A, E5=KAZARDI-Kabale 2018A, E6=Bushenyi2018A, E7= Bulegeni2018A

Table 5. Mean values of 25 rice genotypes evaluated for leaf growth/ leaf wilting score in each the test environment during the 2017B and 2018A growing seasons

Line code	Line name	[1]**E1**	[2]**E2**	[3]**E3**	[4]**E4**	[5]**E5**	[6]**E6**	[7]**E7**
G20	ARC36-2-1-2	6.0	7.8	8.2	7.0	6.0	4.4	4.9
G14	MET P31	2.9	2.0	3.8	1.7	3.0	3.0	1.1
G24	MET P36	4.3	4.0	3.0	1.0	2.3	2.3	2.3
G23	MET P37	4.7	5.0	4.3	2.3	4.0	3.0	1.0
G2	MET P27	3.1	2.1	3.5	1.0	3.0	1.7	0.9
G10	MET P40	2.9	3.5	2.0	1.0	1.0	1.7	1.6
G4	MET P32	2.2	5.0	4.8	3.7	3.9	1.0	2.3
G18	MET P39	3.8	3.4	3.6	4.3	4.0	3.7	3.7
G12	MET P22	3.1	5.0	4.6	3.7	3.7	3.0	3.0
G22	MET P18	4.0	4.0	3.0	2.3	4.3	3.0	2.3
G9	MET P17	2.8	3.2	3.1	2.3	3.0	2.3	3.0
G13	MET P20	1.5	5.0	3.3	1.7	3.3	1.7	3.7
G5	MET P23	2.7	2.0	3.0	1.7	1.7	1.0	0.9
G16	MET P24	4.6	5.7	2.5	2.3	4.0	3.3	2.4
G25	MET P5	4.3	4.7	3.0	1.0	2.3	1.7	2.3
G21	MET P2	2.0	3.7	3.7	1.7	3.7	1.0	1.3
G6	MET P11	2.3	2.2	3.4	3.0	2.3	3.0	3.3
G15	MET P3	3.4	3.6	3.1	1.7	3.6	3.0	1.8
G7	MET P16	3.5	3.2	3.3	2.3	3.4	3.0	1.0
G17	MET P9	4.5	2.5	3.3	2.3	2.4	2.3	1.4
G3	GIZA 177	2.4	3.5	3.8	1.0	1.7	3.0	1.3
G11	NERICA 1	3.8	4.4	2.9	1.0	1.0	1.0	1.0
G8	MET P60	3.6	2.0	1.6	1.0	2.6	1.3	0.9
G1	SCRID091-20-2-2-4-4	3.0	1.9	3.0	1.0	2.6	1.0	1.7
G19	WITA9	5.2	7.0	7.0	7.0	6.7	3.7	5.3
	Mean	**3.5**	**3.9**	**3.6**	**2.4**	**3.2**	**2.4**	**2.2**
	LSD (5%)	**2.0**	**1.7**	**2.0**	**1.4**	**1.6**	**1.4**	**1.2**
	Min	**1.5**	**1.9**	**1.6**	**1.0**	**1.0**	**1.0**	**0.9**
	Max	**6.0**	**7.8**	**8.2**	**7.0**	**6.7**	**4.4**	**5.3**

[1]Zombo2017B, [2]KAZARDI 2017B-Kabale, [3]BZARDI 2017B-Mbale, [4]Zombo 2018A, [5]KAZARDI 2018A-Kabale, [6]Bushenyi 2018A, [7]Buligeni 2018A, LSD= Least significant different

growth scores implying that these genotypes had better tolerance to cold environments. Based on the mean performance across environments, genotypes G5 (MET P23), G1 (SCRID091-20-2-2-4-4), G8 (MET P60), G10 (MET P40) and G21 (MET P2) were the top best five rice genotypes with scores ranging from 1.86 to 2.10. These genotypes were hence categorized as tolerant to cold stress. Genotypes G5 (MET P23), G1 (SCRID091-20-2-2-4-4) and G8 (MET P60) were among the highly cold tolerance genotypes identified under cold growth chamber as well as in field conditions. The performance of genotypes varied from one environment to another as indicated by a high significant variation in GEI. Among all the environments under which the genotypes were evaluated, the genotypes had relatively better performances for leaf growth in E6 (Bushenyi 2018A) followed by E7 (Bulegeni 2018A). The minimum temperature of these areas was higher compared to other environments, which led to better performance for certain genotypes (Table 1). In general, leaf wilting score was one of the major traits on which the symptom of cold stress observed on the rice genotypes evaluated. This trait was, therefore, chosen in this study from seedling stage traits for demonstration of AMMI ANOVA and GGE bi-plot. AMMI analysis showed highly significant environment, genotype and GEI effects on leaf wilting score. The analysis also indicated that the first two principal components (PC1 and PC2) were highly significant (P<0.001) and they explained 72.7% of the total variation of GEI. polygon view of GGE bi-plot indicate (Figure 2). The partitioning of the total sum of squares (TSS) showed that the environmental effect was a predominant source of variation followed by GEI and genotype in the performance of the genotypes evaluated (Table 6). Similarly environments accounted the largest variations followed by genotypes by environments interaction and genotypes in study of (Lemma & Mekbib, 2021) on grain yield of 25 durum wheat genotypes.

(ii). AMMI analysis

The AMMI analyses of variance for the 25 rice genotypes evaluated in seven environments for leaf growth/leaf wilting score are presented in Table 6. Results showed that the highest (26.34%) total sum square variation was attributed to environmental effect followed by interaction effect (23.04%) and only 13.88% was attributed to the genotype effect. The environment sum square (15.47) was about 2 times larger than that of genotype sum square (8.15) indicating that the environment plays a substantial role in genotype variation. On the other hand, GEI was highly significant (p<0.01) for this trait indicating that the rice genotypes responded differently to different environmental condition.

Table 6. AMMI analysis of variance for leaf growth/ leaf wilting score of evaluated rice across seven environments

Source	D.f	S.S	M.S	Explained S.S (%)
Total	524	58.7	0.1	
Genotypes	24	8.2	0.3***	13.9
Environments	6	15.5	2.6***	26.3
Interactions	144	13.5	0.1**	23.0
IPCA 1	29	4.1	0.1***	
IPCA 2	27	3.2	0.1***	
Pooled error	258	294.0	1.1	

, and*significant at probability level of 0.01 and 0.001

(iii). GGE Bi-plot analysis of different environment for leaf growth/leaf wilting score

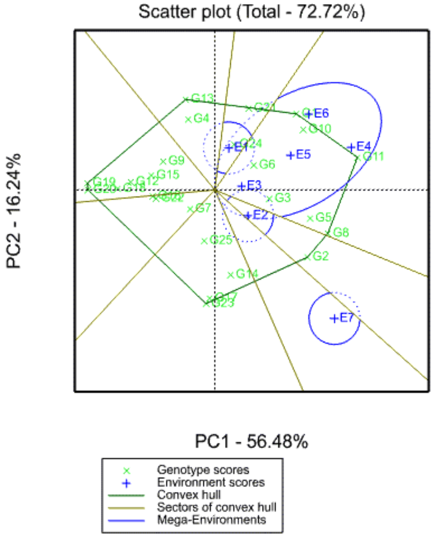

Figure 2. Polygon view of GGE bi-plot for leaf growth/leaf wilting score in rice across seven environments

The polygon view of the 25 tested rice genotypes using leaf wilting score is presented in Figure 2. The first two principal component axes explained 72.72% of the total variation of GEI. From the Figure 3, G1 and G11 were vertex genotypes in environment E3, E4, E5 and E6, indicating that they were the best performing genotypes in these environments. Genotypes G2 and G8 were vertex genotypes in E2 suggesting that they were the best performers in environment E2. The susceptible genotypes (G19 and G20) were on the vertex but did not fall in any the environments indicating their poor performance in all testing environments. The best genotypes identified, G1, G2 and G8 were also among the cold tolerant genotypes identified in the cold growth chamber at seedling stage.

(iv). Seedling color /leaf yellowing score

Results from the ANOVA showed that the evaluated rice genotypes were varied significantly (P<0.01 and P<0.001) for seedling color score in all tested environments except in E1 and E4 (Table 4). Seedling color scores were used to evaluate the performance of the genotypes for seedling color. The genotypes were categorized into five seedling color scores: dark green (1.0 to 1.5), light green (1.51 to 3.50), yellowish (3.51 to 5.50), reddish brown (5.51 to 7.5) and dead (7.51 to 9.0). Very few rice genotypes were observed to have dark seedling color in E1 and E3 and the seedlings appeared mostly as light green and yellowish. None of the rice genotypes had dark green seedling color in E2 and most of the genotypes had light green to yellowish seedling color. In contrast, a high number of rice genotypes (10) with dark seedling color was observed in E4, followed by E7 (8 genotype) and E6 (5 genotypes) (Figure 3). Using the visual score on the color of seedlings, genotype G4 had dark green seedling color in E1 and E6 with a score of 1.3 and 1.0, respectively. Similarly, genotype G6 with the lowest seedling color score of 1.6 in E2 was categorized as light green. Meanwhile, genotypes G8 in E3, G10 in E4, G3 in E5, and G2 in E7, had a seedling color score of 1.0 in their respective environments. Genotypes G19 and G20 showed yellowish to reddish brown colors in most of the test environments (Table 7). Leaf yellowing (the visible

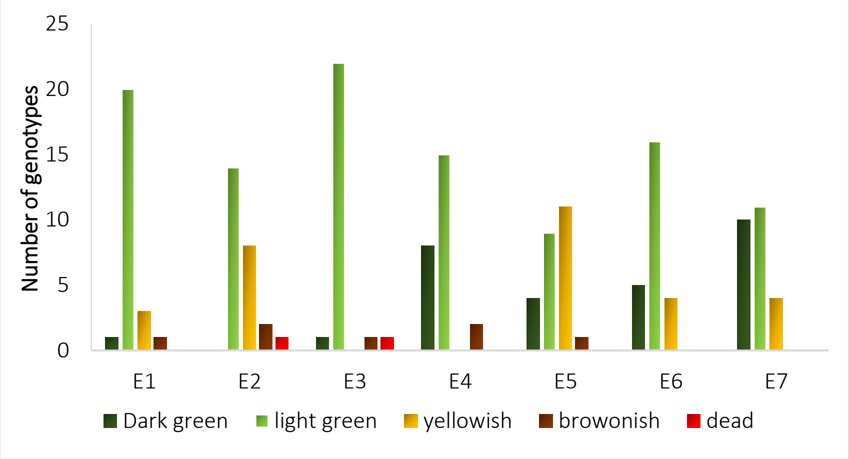

Figure 3. Frequency distribution of selected rice genotypes for seedling color/leaf yellowing score in seven environments evaluated at seeding stage during the 2017B and 2018A growing seasons

Where: E1=Zombo2017B, E2=KAZARDI-kabale 2017B, E3=BZARDI-Mbale 2017B, E4=Zombo 2018A, E5=KAZARDI-Kabale 2018A, E6=Bushenyi2018A, E7= Bulegeni2018A loss of chlorophyll content) was determined by seedling color/ leaf yellowing score developed by IRRI (2013). The visible yellowing of rice seedling is due to low temperature, which blocks the electron transfer from PSII (photosystem two) to PSI (photosystem one) and degrading the PSII complex. In other words, leaf yellowing is a result of complete destruction of PSII and PSI complexes in which chlorophyll is almost lost (Suzuki et al., 2008). It has been reported in many

Table 7. Mean values of 25 rice genotypes evaluated for seedling color score in each the test environment during the 2017B and 2018A growing seasons

Line code	Line name	[1]E1	[2]E2	[3]E3	[4]E4	[5]E5	[6]E6	[7]E7
G20	ARC36-2-1-2	5.7	7.6	8.2	6.2	7.6	3.0	5.0
G14	MET P31	1.7	2.4	2.6	1.7	2.4	3.6	1.0
G24	MET P36	2.3	3.0	1.7	1.0	3.0	1.7	2.3
G23	MET P37	3.7	5.0	3.0	3.0	5.0	3.0	1.0
G2	MET P27	3.1	2.2	1.9	1.6	2.2	1.8	1.0
G10	MET P40	1.7	2.9	1.8	1.0	2.9	1.8	1.7
G4	MET P32	1.3	4.2	3.5	3.1	4.2	1.0	2.3
G18	MET P39	2.8	3.8	3.1	3.0	3.8	3.6	3.7
G12	MET P22	2.7	4.4	3.3	4.3	4.4	3.0	3.0
G22	MET P18	3.3	3.3	2.3	2.3	3.3	3.0	2.3
G9	MET P17	1.8	2.7	2.3	2.4	2.7	3.0	2.3
G13	MET P20	1.7	4.2	2.8	1.7	4.2	1.8	3.7
G5	MET P23	2.9	2.4	1.7	1.6	2.4	1.0	1.0
G16	MET P24	4.0	6.4	2.0	2.3	6.4	3.6	2.3
G25	MET P5	3.0	3.7	1.7	1.0	3.7	1.7	2.3
G21	MET P2	1.7	3.3	3.0	1.7	3.3	1.0	1.0
G6	MET P11	2.4	1.6	1.8	2.9	1.6	3.1	3.0
G15	MET P3	2.4	3.3	2.3	1.1	3.3	3.0	1.7
G7	MET P16	3.1	4.0	2.8	1.7	4.0	2.5	1.0
G17	MET P9	2.9	2.4	2.6	1.6	2.4	2.3	1.7
G3	GIZA 177	2.3	3.4	3.5	1.0	3.4	2.4	1.0
G11	NERICA 1	2.8	4.4	3.1	1.0	4.4	1.0	1.0
G8	MET P60	3.0	2.7	1.0	1.2	2.7	1.7	1.0
G1	SCRID091-20-2-2-4-4	2.4	1.6	2.3	1.1	1.6	1.0	1.3
G19	WITA9	4.4	7.5	5.9	6.4	7.5	3.6	5.0
	Mean	**2.8**	**3.7**	**2.8**	**2.2**	**3.7**	**2.3**	**2.1**
	LSD (5%)	**Ns**	**2.2**	**2.0**	**Ns**	**2.0**	**1.7**	**1.2**
	Min	**1.3**	**1.6**	**1.0**	**1.0**	**1.6**	**1.0**	**1.0**
	Max	**5.7**	**7.6**	**8.2**	**6.4**	**7.6**	**3.6**	**5.0**

[1]Zombo2017B, [2]KAZARDI 2017B-Kabale, [3]BZARDI 2017B-Mbale, [4]Zombo 2018A, [5]KAZARDI 2018A-Kabale, [6]Bushenyi 2018A, [7]Buligeni 2018A, LSD= Least significant different and ns = non-significant

Table 8. Mean values of top five and bottom five selected rice genotypes for survival rate in each the test environment during the 2017B and 2018A growing seasons

Survival rate (%)													
[1]E1		**[2]E2**		**[3]E3**		**[4]E4**		**[5]E5**		**[6]E6**		**[7]E7**	
Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean
G8	85.6	G1	80.4	G10	84.5	G1	85.2	G10	90.0	G10	92.7	G10	84.3
G7	83.6	G8	78.9	G8	79.5	G10	84.4	G8	87.5	G8	90.0	G8	82.2
G1	76.3	G5	77.1	G1	76.5	G25	83.2	G5	86.3	G1	87.0	G1	80.7
G10	75.7	G2	76.8	G5	76.4	G11	83.0	G6	83.1	G2	85.4	G3	78.4
G5	70.7	G10	75.8	G17	75.9	G2	82.4	G1	83.1	G6	84.4	G14	78.1
G25	46.7	G22	60.7	G23	64.2	G23	66.7	G18	70.8	G15	66.8	G22	66.0
G4	45.6	G18	54.4	G22	60.6	G12	63.4	G6	70.1	G21	64.6	G18	63.2
G21	40.0	G13	53.8	G4	59.8	G2	52.1	G12	68.4	G16	64.0	G13	59.7
G23	40.0	G20	26.6	G19	46.4	G19	45.5	G20	45.0	G19	52.7	G20	53.7
G19	39.9	G19	15.0	G20	42.0	G20	42.3	G19	39.3	G20	46.3	G19	52.1
Mean	**58.4**		**64.7**		**68.1**		**71.5**		**71.5**		**75.2**		**70.9**
LSD (5%)	**15.3**		**30.5**		**15.7**		**10.4**		**13.6**		**9.0**		**15.3**

[1]Zombo2017B, [2]KAZARDI 2017B-Kabale, [3]BZARDI 2017B-Mbale, [4]Zombo 2018A, [5]KAZARDI 2018A-Kabale, [6]Bushenyi 2018A, [7]Buligeni 2018A, LSD= Least significant different

studies that low temperature affects the chlorophyll content, consequently affecting the photosynthetic process (Kura Hotta et al., 1987; Roy and Challa, 2012; Hasani et al., 2013; Adamski et al., 2016). In the current study, significant variations were observed among the genotypes evaluated for seedling colors scores in all of the test environments except in E1 (Zombo 2017B) and E4 (Zombo 2018A). The rice genotypes were categorized into five seedling colors based on the mean score (i.e Dark green, light green, yellowish, few brownish and very few died) (Table 6 and Figure 2). A high number of dark green seedling colors were scored for E7 (Bulegeni, 2018A) which could be attributed to the relatively high minimum temperatures ranging from 18^{o}C-19^{o}C during seedling growth (Table 1). In contrast, E2 (Kabale, 2017B) and E5 (Kabale, 2018A) experienced low temperatures (10 ^{o}C) which resulted into yellowish seedling color in most of genotypes. This result was similar to the findings reported by Roy and Challa (2012), in which genotypes were evaluated in three environments, of which the two environments experienced low temperature and one had better temperature. Genotypes had better seedling in environments with high temperatures than the two environments with low temperature. The GEI was highly significant indicating genotypes response differently to different environmental variation.

(v). Survival rate (%)

The evaluated rice genotypes were significantly different (P<0.01 and P<0.001) in their response to cold calculated as survival rate (%) in all test environments (Table 4). Table 8 summarizes the mean percentage survival rate of the top five and the bottom five rice genotypes evaluated in seven environments. Based on individual environment survival rate performance, genotype G8 had the highest (85.5%) survival rate in E1; G1 both in E2 and E3 at a survival rates of 80.4% and 85.2% respectively, G10 in E3, E5, E6 and E7 with survival rates of 84.5%, 90%, 92.7% and 84.5% respectively. On the other hand, genotypes G19 and G20 showed the lowest survival rates, with values ranging from 15% to 53.7% in all the test environments in which they were tested (Table 8). The survivability of seedlings is crucial to attain optimum plant population in field (Kazemitabar et al., 2003). Seedling survival percentage is a useful trait for addressing the differences between greenhouse and field based results and it is the most practical cold tolerance evaluation criteria as seedlings usually experience cold stress in the field especially in highland areas (Zhang et al., 2014; Lou et al., 2007). The rice genotypes evaluated in the current study showed high significant variation for survival rate in all the test environments. These could be attributed to the existence of genetic variability among the genotypes for survival under cold environments. The GEI effect had no significant effect on genotype performance, suggesting consistence performance of genotypes for survival rate across environment. Based on combined mean performance across environment, G10 (MET P40) (82.94%) had high survival rate followed by G8 (MET P60) (81.61%) and G1 (SCRID091-20-2-2-4-4) (81.32%) (Table 10). On the other hand, genotype G20 (ARC36-2-1-2) with a survival rate of 40.75% was reported to be a poor performing genotype across environments.

(vi). Seedling height (cm)

The rice genotypes evaluated in this study were significantly different (P<0.05, p<0.01 and P<0.001) for seedling height in all test environments in which they were evaluated except in E7 (Table 4). Table 9 shows the mean seedling height for top five and bottom five genotypes evaluated in seven environments. The tallest (50.2 cm) rice genotype in E1 was G17. Similarly, G16 (24.4 cm) in E2; G1 (39.0 cm) in E3; G5 (55.1 cm) in E4; G10 in E5 and E7 with mean height of 40.6 cm and 51.7 cm respectively, and G2 (55.3 cm) in E6 were the tallest genotypes in the respective environments. Genotype G19 was the shortest genotype in all the tested environments with seedling height ranging between 13.8 to 28.8 cm, except in E2 and E3 where G3 and G20 were the shortest rice genotypes with seedling height of 15.5 cm and 17.4 cm, respectively. Among the testing environments, relatively high mean seedling height was recorded in E7 followed by E4 with the grand mean of 45.8 cm and 45.3cm, respectively. The lowest (21.0 cm) mean seedling height was recorded in E2. Stunting of rice seedling is one of cold stress effects reported in many studies (Andaya and Mackill, 2003; Kim et al., 2012; Gothandam, 2012). Therefore, seedling height can be a good

Table 9. Mean values of top five and bottom five selected rice genotypes for seedling height in each the test environment during the 2017B and 2018A growing seasons

Seedling height													
^{1}E1		**^{2}E2**		**^{3}E3**		**^{4}E4**		**^{5}E5**		**^{6}E6**		**^{7}E7**	
Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean	Line	Mean
G17	50.2	G16	25.4	G1	39.0	G5	55.1	G10	40.6	G2	55.3	G10	51.7
G7	47.0	G4	24.3	G16	38.2	G1	54.4	G24	40.2	G16	53.2	G23	50.8
G5	46.7	G1	24.1	G2	37.0	G7	52.6	G15	39.9	G17	52.9	G17	49.9
G1	45.6	G17	23.8	G9	36.9	G21	52.4	G16	38.5	G10	52.5	G15	49.8
G10	45.3	G2	23.5	G4	34.6	G2	51.0	G4	38.0	G1	52.5	G14	49.7
G24	34.8	G24	18.3	G13	27.9	G12	41.4	G6	30.3	G22	36.8	G3	39.6
G25	29.3	G13	18.1	G25	27.8	G25	40.9	G23	28.6	G18	36.4	G25	38.5
G3	24.8	G19	17.2	G3	25.2	G3	37.0	G12	28.6	G3	35.7	G9	37.5
G20	23.8	G20	16.7	G19	21.2	G20	26.5	G20	21.7	G20	27.9	G20	29.7
G19	16.4	G3	15.5	G20	17.4	G19	18.4	G19	13.8	G19	19.4	G19	28.8
Mean	**39.1**		**21.0**		**30.6**		**45.3**		**33.4**		**42.7**		**45.8**
LSD (5%)	**10.0**		**5.4**		**6.2**		**16.1**		**8.4**		**14.9**		**Ns**

^{1}Zombo2017B, ^{2}KAZARDI 2017B-Kabale, ^{3}BZARDI 2017B-Mbale, ^{4}Zombo 2018A, ^{5}KAZARDI 2018A-Kabale, ^{6}Bushenyi 2018A, ^{7}Buligeni 2018A; ns= non-significant, LSD= Least significant different parameter in evaluating rice seedling for cold tolerance. Significant variation was observed among genotypes in their responses to seedling height in all environments except in E7 (Bulegeni 2018A), which imply the existence of genetic variation among evaluated genotypes. The effect of GEI was also significant for this trait, suggesting inconsistence performance among genotypes. Based on individual mean comparisons, the lowest mean seedling heights were recorded for environment E2 (KAZARDI-Kabale 2017B) followed by E3 (BZARDI-Mbale 2017B) and E5 (KAZARDI-Kabale 2018A). The highest mean seedling heights were recorded in E7 (Bulegeni 2018A) followed by E4 (Zombo 2018A), E6 (Bushenyi 2018A) and E1 (Zombo 2017B) (Table 9). Metrological data showed that E2, E3 and E5 environments had lower minimum temperatures compared to environments E1, E4, E6 and E7 which had relatively higher minimum temperatures (Table 1). Therefore, it is possible that temperature variations observed in the different environments accounted for the observed seedling height reductions. Similar result was reported by Roy and Challa (2012) in which seedling height was significantly reduced in environments where temperatures dropped below 15°C and

Table 10. Pooled analysis of variance for GEI for different seedling stage traits of rice

Source of variation	**[8]D.f**	**[10]LG**	**[11]SC**	**[12]%SR**	**[13]SV**	**[14]SH**
Total	174	2.2	2.0	192.3	2.9	179.3
[1]E	6	11.9*	8.2**	1708.02^{ns}	17.4*	3294***
[2]Rep/E	14	3.0ns	1.8^{ns}	972.5	6.1**	101.1^{ns}
[3]BLK/(Rep, E)	84	2.8****	0.8^{ns}	306.9***	2.3**	62.7*
[4]G	24	25.4***	23.1***	21557.2***	33.4***	359.3**
[5]GEI	144	2.1***	2.3***	124.0^{ns}	2.7***	178.5***
Pooled error	253-290	1.1	1.3	121.5	1.48	42.8
[6]SED		**0.6**	**0.6**	**5.9**	**0.7**	**3.5**
[7]CV		**35.6**	**41.4**	**20.3**	**32.9**	**16.9**

ns= non-significant, *, **, and *** significant at probability level of 0.05, 0.01 and 0.001 respectively, [1]Environment, [2]Replication nested in environment, [3]Block nested in replication and environment, [4]Genotype, [5]Genotype by environment interaction, [6]Standard error difference, [7]Coefficients of variation, [8]Degree of freedom, [9]%SE= Seedling emergence percentage, [10]Leaf growth, [11]Seedling color, [12]Survival rate, [13]Seedling vigor, [14]Seedling height and [15]Tiller number

Table 11. Combined means of 25 rice genotypes for seedling stage traits

Line code	**Line name**	**[2]LG**	**[3]SC**	**[4]SV**	**[5]%SR**	**[6]SH**
G20	ARC36-2-1-2	6.3	5.8	7.1	41.5	32.5
G14	MET P31	2.5	2.4	3.3	73.9	38.3
G24	MET P36	2.8	2.1	3.5	67.8	39.0
G23	MET P37	3.5	3.3	3.6	66.4	38.2
G2	MET P27	2.2	2.1	3.0	76.0	45.0
G10	MET P40	2.0	1.7	2.4	85.1	42.8
G4	MET P32	3.3	2.8	3.6	65.8	42.6
G18	MET P39	3.8	3.5	4.3	65.9	39.7
G12	MET P22	3.7	3.4	4.6	66.0	37.6
G22	MET P18	3.3	2.9	4.1	68.2	37.6
G9	MET P17	2.8	2.5	3.3	71.9	40.1
G13	MET P20	2.9	2.8	3.6	72.6	38.8
G5	MET P23	1.9	1.7	2.3	78.8	40.9
G16	MET P24	3.5	3.6	3.9	67.8	43.5
G25	MET P5	2.8	2.2	3.8	69.3	36.9
G21	MET P2	2.4	2.2	3.0	65.7	42.8
G6	MET P11	2.8	2.5	3.5	72.0	38.3
G15	MET P3	2.9	2.7	3.8	70.3	40.6
G7	MET P16	2.8	2.7	3.7	76.7	40.2
G17	MET P9	2.7	2.2	4.1	78.4	38.1
G3	GIZA 177	2.4	2.1	4.1	76.7	31.1
G11	NERICA 1	2.2	2.0	3.3	74.7	36.6
G8	MET P60	1.9	1.9	2.0	83.9	39.8
G1	SCRID091-20-2-2-4-4	2.0	1.8	2.1	83.2	41.4
G19	WITA9	6.0	5.7	7.5	44.7	25.7
	Mean	**3.0**	**2.7**	**3.7**	**70.5**	**38.7**
	LSD (5%)	**0.7**	**0.7**	**0.7**	**8.7**	**4.0**
	Min	**1.9**	**1.7**	**2.0**	**41.5**	**25.7**
	Max	**6.3**	**5.8**	**7.5**	**85.1**	**45.0**

[1]%Seedling emergence percentage, [2]Leaf growth, [3]Seedling color, [4]Survival rate, [5]Seedling vigor, [6]Seedling height, [7]Tiller number, LSD= Least significant difference, ns= non-significant, Min= Minimum, Max=Maximum

it took 36 days for seedlings to reach transplanting size, compared to the 30 days period taken by the seedlings to attain transplanting size in environments with optimum temperature. Across environment mean performance revealed G2 (MET P27) (45.0 cm) as the tallest genotype followed by G16 (MET P24) (43.5 cm) and G10 (MET P40) (42.8 cm) (Table 11). MET P27 was among the best cold tolerant genotypes selected under cold growth chamber. The genotype also performed consistently better for seedling height in field cold stress. In contrast, G19 (WITA 9) and G20 (ARC36-2-1-2) were the shortest genotypes observed at both field and cold growth chamber, and hence had poor performance in both stress conditions.

Combined analysis of variance and mean performance of genotypes for seedling traits across environments

Combined analysis of variance in Table 10 for the rice seedling stage traits (namely leaf growth, seedling color, survival rate, seedling vigor and seedling height) revealed that the three components of phenotypic performance (genotype, environment and GEI) were significant (P<0.05, p<0.01 and

Table 12. Pooled analysis of variance for yield and yield component traits across seven environments

SOV	[8]D.f	[9]DTH	[10]Pan EX	[11]PH	[14]TTN	[15]DTM	[16]GY/ha	[17]GFR
Total	159-174	1374.8	2.9	271.6	25.8	1.8	554520	0.021
[1]E	2-6	26074.3***	22.1**	4103.1***	524.5***	3666.8**	1915238.0^{ns}	0.02^{ns}
[2]Rep/(E)	6-14	150.3^{ns}	4.9*	52.3^{ns}	34.0*	101.3^{ns}	332468.9^{ns}	0.008^{ns}
[3]BLK/(Rep, E)	36-84	214.6***	2.5*	50.0^{ns}	19.1***	190.6^{ns}	198913.6^{ns}	0.02**
[4]G	24	1928.7***	24.3***	2128.3***	54.9***	778.8***	3438045***	0.13***
[5]GEI	144	273.3***	2.3***	112.2***	18.3***	172.6^{ns}	606225.0^{ns}	0.03**
Pooled error	253-290	108	1.8	61.5	10.7	160.9	436600.5	0.01
[6]SED		**5.6**	**0.7**	**4.2**	**1.7**	**6.8**	**353.2**	**0.06**
[7]CV (%)		**7.1**	**28.7**	**11.8**	**22.9**	**8.0**	**31.9**	**17.9**

$^{*, **,}$ and***significant at probability level of 0.05, 0.01 and 0.001 respectively, ns= non-significant; [1]environment, [2]Replication nested in Environment, [3]Block nested in replication and environment, [4]Genotype, [5]Genotype by environment interaction, [6]Standard error difference, [7]coefficients of variation, [8]Degree of freedom [9] Days to 50% heading, [10]Panicle exsertion, [11]Plant height, [12]flag leaf length, [13]Flag leaf width, [14]Total tiller number, [15]Days to maturity, [16] Grain yield per hectare and [17]grain filling ratio.

Table 13. Combined means of 25 rice genotypes for yield and yield component traits

Code	**Line name**	**DTH**	**Pan EX**	**PH**	**TM**	**GY(kg/ha)**	**FR**
G20	ARC36-2-1-2	40.3	2.7	0.5	77.2	1581.9	0.0
G14	MET P31	47.4	4.4	9.6	55.5	1917.8	0.0
G24	MET P36	53.6	3.6	2.0	72.1	1801.9	0.0
G23	MET P37	26.0	4.9	2.2	50.3	1888.9	0.0
G2	MET P27	45.2	5.2	0.3	61.7	1771.6	0.0
G10	MET P40	17.6	7.2	3.0	50.6	2984.6	0.0
G4	MET P32	40.0	3.3	2.5	64.5	1713.4	0.0
G18	MET P39	50.1	4.4	6.9	73.7	1898.3	0.0
G12	MET P22	50.0	4.4	4.7	57.7	2142.3	0.0
G22	MET P18	38.3	5.0	4.0	55.0	1736.1	0.0
G9	MET P17	48.5	4.8	9.0	53.8	2320.8	0.0
G13	MET P20	46.9	5.5	0.1	64.5	1802.2	0.0
G5	MET P23	45.4	4.7	6.1	64.8	1804.5	0.0
G16	MET P24	39.7	4.5	6.4	53.5	1827.1	0.0
G25	MET P5	44.0	4.1	2.1	67.1	1872.2	0.0
G21	MET P2	42.1	4.4	7.3	57.3	2137.0	0.0
G6	MET P11	47.6	4.8	2.9	62.0	1769.8	0.0
G15	MET P3	36.2	4.9	9.3	49.2	1857.0	0.0
G7	MET P16	43.7	4.7	1.8	62.8	2149.0	0.0
G17	MET P9	36.6	6.0	6.5	49.1	3242.7	0.0
G3	GIZA177	36.6	5.5	8.1	42.4	2416.4	0.0
G11	NERICA 1	35.2	5.7	2.6	46.0	2546.4	0.0
G8	MET P60	49.8	5.6	0.8	63.0	2257.9	0.0
G1	SCRID091-20-2-2-4-4	30.7	6.4	9.7	45.0	3810.2	0.0
G19	WITA9	52.9	2.3	9.2	85.9	531.2	0.0
	Mean	**41.8**	**4.8**	**6.7**	**59.4**	**2071.2**	**0.0**
	LSD (5%)	**0.9**	**1.4**	**.3**	**3.4**	**699.2**	**0.0**
	Min	**17.6**	**2.3**	**9.2**	**42.4**	**531.2**	**7.8**
	Max	**53.6**	**7.2**	**0.3**	**85.9**	**3810.2**	**4.1**

N.B: DTM and GY/ha were computed for three environments, Days to heading, [2]Panicle exsertion, [3]Plant height, [4]Flag leaf length, [5] Flag leaf width, [6]total tiller number, [7]Grain yield kg per hector, [8]Grain filling ration, LSD = Least significant difference, Min=Minimum, Max=Maximum

P<0.001) except environment and GEI was not significant for survival rate. A wide range of variation was observed among the rice genotypes evaluated based on across environment seedling stage traits performance. Rice genotypes, G5, G8 and G10 were the top three for leaf wilting trait with mean scores of 1.85, 1.87 and 1.95, respectively. Genotypes G20 and G19 had high leaf wilting mean scores (6.33 and 5.98) respectively. Based on the color scores of seedlings, G40, G5 and G1 were the top three genotypes with score of 1.69, 1.71 and 1.81, respectively. Genotypes G19 and G20 had the highest score with mean of 5.66 and 5.76, respectively. The most vigorous rice genotypes across environment was G8 (1.99) followed by G1, G5 and G10 with score of 2.05, 2.26 and 3.37, respectively. G19 and G20 were the most stunted and weakest rice genotypes according to this trait. Highest (85.13%) survival rate across environments was recorded for G10 followed by G8 (83.93%) and G1 (78.78%). The lowest survival rate was recorded for G20 (41.48%) followed by G19 (44.96%) (Table 11).

Performance of selected rice genotypes for yield and yield component traits

Mean square of yield and yield component traits are presented in the Table 12. Most of the genotypes showed significant (P<0.05, p<0.01 and P<0.001) differences for the evaluated genotypes across the different environments.

Combined analysis and mean performance of rice genotypes across environments in yield and yield component

Combined analysis of variance for yield and yield components of the 25 rice genotypes evaluated across environments is presented in Table 13. The results revealed highly significant Genotype environments interaction (GEI) for all the characters evaluated including days to 50% heading, panicle exsertion, plant height, flag leaf length, flag leaf width, tiller number and grain filling ration. Results for grain yield and days to maturity indicated non-significant GEI for the rice genotypes evaluated across environment. Combined analysis of variance also showed significant (P<0.01 and P<0.001) variation among rice genotypes and environments for all traits except environments were not significant for grain yield and grain filling ratio (GFR). The significant variation among environments reflected considerable differences among the test environments. The combined analysis of variance showed that genotypes, environments and GEI were highly significant for days to 50% heading. Based on means of across environments, the earlier genotypes for days to 50% heading were G10 (117.6 days) followed by G23 (126 days), G1 (140 days) and G11 (135.2 days), whereas G24 (153.6) was the late genotype to achieve 50% heading (Table 13). Several studies have reported studies that low temperatures delay heading in rice (Datta, 1981; Yoshida, 1981; Cruz *et al*., 2008; Shantanu *et al*., 2015). A temperature drops by 1°C from the optimum was reported to cause 13 days delay in heading (Yoshida, 1981). Yoshida, 1981 study revealed that an increase of temperature from 21°C to 24 °C resulted in a decrease in the number of days to heading from 134 to 96. In the current study, rice genotypes were significantly different in their responses to days to heading indicating variation in performance among the genotypes evaluated. In addition, environment and GEI were significantly variable, implying the difference in performance of genotypes from environment to environment. The lowest duration to 50% heading across environment was recorded on G10 (MET P40) (117.6 days) followed by G23 (MET P37) (126 days) and G1 (SCRID091-20-2-2-4-4) (130.7 days) (Table 13). This implies that these rice genotypes had the ability to achieve 50% heading earlier than the others, which made them to be more tolerant to the cold stress across the environments. Combined analysis of variance showed that genotypes, environment and GEI were highly significant for panicle exsertion (Table 12). Based on the mean performance across environments, genotype G10 had better panicle exsertion followed by G1, G17, G11 and G3. Poor panicle exsertion was recorded for G19 and G20 (Table 13). Incomplete panicle exsertion is one of the symptoms of cold injury at reproductive stage in rice, which reduces grain yield, and it is a better indicator of cold tolerance under field conditions (Datta, 1981; Lee, 2001; Cruz *et al*., 2008). The significant GEI indicated that the rice genotypes responded differently to the different environmental conditions. Panicle exsertion was negatively affected by cold stress in a study conducted by Ndour *et al*. (2016). However, in the current study, some rice genotypes were able to tolerate the cold stress and showed good panicle exsertion. Genotypes G10 (MET P40) followed by G11 (NERICA 1), G1 (SCRID091-20-2-2-4-4) and G3 (GIZA 177), were well exerted in E2 (KAZARDI-Kabale 2017B) which were also performed well across all environments. These genotypes were among the best cold tolerant lines identified in cold growth chamber as well under field cold conditions. Genotypes, environments and GEI were highly significant (P<0.001) for plant height (Table 12). Stuntedness is one of the cold stress symptoms according to several reports (Yoshida, 1981; Lee, 2001; Ndour et al., 2016; Pradhan and Rani, 2017). The rice genotypes evaluated in this study showed significant variations in their responses to plant height, indicating the existence of genetic differences among them. The GEI was highly significant for this trait, indicating phenotypic expression for plant height was variable among the genotypes tested in different environments. Combined mean comparisons revealed that genotypes G2 (MET P27) followed by G21 (MET P2), G5 (MET P23) and G10 (MET P40) were the tallest among genotypes evaluated. The shortest plant height was recorded on genotypes G19 (39.2 cm) and G20 (40.5 cm) (Table 13). These rice genotypes were also among the best cold tolerant genotypes reported under the cold growth chamber evaluation, suggesting their consistent performance in the controlled and field cold stress conditions.
Combined analysis of variance for days to maturity for the three environments revealed highly significant variation among the rice genotypes across environments (Table 12). GEI was not significant for this trait. Based on across mean analysis, genotype G3 (142.4 days) was reported to be the earliest maturing genotype followed by G1 (145.0 days) and G17 (146.0 days). The longest duration to 85 % physiological maturity was recorded on genotype G20 (Table 13). Cold stress delayed crop maturity by lengthening the maturation duration. Many rice growers prefer early maturing varieties (Sié et al., 2013). In the current study, GEI effect was not significant on days to 85% physiological maturity, suggesting

the consistent performance of genotypes evaluated across environments. However, there was a significant variation among genotypes indicating the existence of genetic diversity among the genotypes evaluated (Table 12). Results of the means across environments showed that G3 (GIZA 177), G1 (SCRID091-20-2-2-4-4), G11 (NERICA 1), G17 (MET P9) and G10 (MET P40) were the five early to late maturing genotypes (Table 13). General performance of these genotypes for seedling and some other yield component traits were also relatively good. The first three genotypes (i.e G3, G1 and G11) reported as good performing genotypes in this study were also reported as cold tolerant genotypes in other studies. G1 (SCRID091-20-2-2-4-4) was reported as cold tolerant in the screening conducted in Uganda (Nyiramugisha *et al*., 2017), G3 (GIZA177) in (*Suh et al.*, 2013), and G11 (NERICA 1) in (Dessie et al., 2014; Wainaina et al., 2015).

The combined mean computed for grain yield in the three environments showed significant differences among the rice genotypes evaluated, whereas the environments and the GEI effects were not significant (Table 12). Based on across environment means, genotype G1 (3810.2 kg/ha) was the highest yielding followed by G9 (3242.7kg/ha), G10 (2984.6kg/ha) and G3 (2546.4 kg/ha). The lowest yielding rice genotype was G19 (531.2 kg/ha) followed by G20 (1584.9kg/ha) (Table 13). Yield performance of individual genotypes in a given environment reflects on the cumulative environmental effects on the different processes involved in building the final yield. Temperatures affect rice yield by affecting tillering, spikelet formation and ripening (Yoshida, 1981). In the current study, significant variation was observed among the genotypes evaluated for grain yield, indicating the presence of genetic diversity. Ghadirnezhad and Fallah (2014) also found significant variation among genotypes for grain yield in the study conducted on the effect of temperature on yield and yield component of different rice cultivars. Environment and GEI had no significant effect on the performance of genotypes, implying that the observed yield performances were mainly due to the genetic effect of the genotypes. Based on means across environments, high yielding genotypes were recorded from G1 (SCRID091-20-2-2-4-4) followed by G17 (MET P9), G10 (MET P40), G11 (NERICA 1), and G3 (GIZA177). These genotypes were early maturing and were among the best cold tolerant genotypes identified both in the field and under the cold growth chamber. The low yielding genotype across environments was G19 (WITA9). These genotypes were susceptible to cold stress in both field and cold growth chamber evaluation.

High grain filling ratio was recorded on genotypes G1, G40, G17 and G11 in which 80% of harvested yield were filled grain. A low percentage of grain filling was recorded on genotype G19 in which only 20% of harvested yield was filled grain (Table 13). Too cold temperatures adversely affect rice at reproductive and grain filling stages (Pradhan and Rani, 2017; Ndour et al., 2016). The GEI effect on the genotypes evaluated was significant indicating that the trait was highly influenced by environmental variation. Grain filling percentage of the genotypes evaluated ranged from 20% for G19 (WITA 9) to 80% for G1 (SCRID091-20-2-2-4-4), G11 (NERICA 1), G17 (MET P9) and G10 (MET P40) (Table 13). The lowest grain filling percentage indicated that the harvested yield was mostly from sterile spikelet exhibited by the susceptibility of the genotypes to cold conditions. On the other hand, the highest grain filling percentage indicated that the harvested yield was mostly from filled grain, suggesting the tolerance of the genotypes to the stress conditions. Yoshida (1981) reported that, subjecting rice plants to below 20°C close to the reduction division stage of pollen mother cells usually induces a high percentage of spikelet sterility resulting in low grain filling ratio. In a study conducted by Shrestha (2012), cold sensitive rice genotypes had lower grain filling at temperatures of 19 ^{o}C on genotypic responses of upland rice to an altitudinal gradient and 100% sterility at temperature of 13^{o}C.

CONCLUSION

In general, the rice genotypes evaluated in this study showed significant variation in their responses to the traits of interest, in individual and across environments, indicating the existence of significant genetic variation in these genotypes. A significant variation in genotype by environment interaction (GEI) suggested that the genotypes evaluated had inconsistent performance across environments, under which they were evaluated. Meteorological data revealed existence of low temperatures in the test environments. However, there were some genotypes that performed better under these situations. This indicates presence of potential cold tolerance rice genotypes among the materials evaluated. The best performing rice genotypes identified under cold growth chamber were G1 (SCRID091-20-2-2-4-4), G11 (NERICA1), G10 (MET P40), G17 (MET P9) and G3 (GIZA 177) which consistently performed well under field conditions for most of the traits examined, including yield, across environments. Therefore, these genotypes can be used as potential sources of genetic material in breeding for cold tolerance in rice. Temperature at KAZARDI-Kabale and BZARDI-Mbale were very low especially for reproduction and thus not conducive for screening. Genotypes in these areas performed poorly and took longer to achieve 50% heading thus delaying maturity. Crop growth extended into the dry season resulting into poor grain filling. These environments therefore need highly cold tolerant and early maturing rice genotypes. Thus, in cold tolerance rice breeding programs, earliness should be a priority trait in both parental and breeding line selection.

AUTHOR CONTRIBUTIONS

Kidist Tolosa was designed the 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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COMPETING INTERESTS

The authors have no conflict of interests.

ETHICS APPROVAL

Not applicable

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