

Research Application Summary

Response to cold stress at reproductive stage of introduced and adapted rice genotypes in Uganda

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Abstract

Exposure of rice (*Oryza sativa*) to cold stress has significant negative impacts on its development and yield. Prospects of increasing production by growing rice in highland areas of Uganda have not been fruitful due to cold stress. It is therefore important to assess the performance of rice varieties to low temperature to identify germplasm that are cold tolerant with a view of growing them in highland Uganda. The goal of this study was to determine the reaction of a collection of rice accessions for tolerance to cold stress. Fifty rice cultivars that included exotic and locally adapted genotypes were evaluated for two seasons, namely, the second season of 2015 and the first season of 2016. These genotypes were planted under normal environmental conditions and later exposed to 17°C for 30 days when booting began. Data were collected on tiller number, plant height, panicle number, panicle exertion and spikelet fertility, using standard procedures. The results revealed significant ($P < 0.001$) differences between genotypes. In addition, a significant relationship ($r = 0.91$, $P < 0.001$) was observed between panicle exertion and spikelet fertility, indicators for cold tolerance at reproductive stage. Low temperature tolerant genotypes were identified. This then calls for further testing in highland regions of Uganda. However, the identified cold tolerant varieties are short grain yet consumers in the region prefer long grain rice. Thus, there is need to incorporate cold tolerance into the preferred varieties.

Key words: Accessions, cold tolerant, germplasm, *Oryza sativa*, Uganda

Resume

L'exposition du riz (*Oryza sativa*) au stress du froid a des impacts négatifs importants sur son développement et son rendement. Les perspectives d'accroissement de la production par la culture du riz dans les régions montagneuses de l'Ouganda n'ont pas été fructueuses en raison du stress dû au froid. Il est donc important d'évaluer la performance des variétés de riz à basse température à différents stades de croissance afin d'identifier les germoplasmes qui sont tolérants au froid dans le but de les cultiver dans les hautes terres en Ouganda. L'incorporation de traits tolérants au froid dans des lignes adaptables est la stratégie la plus idéale et prometteuse pour développer des variétés de riz à cet effet. Le but de cette étude était de déterminer la réaction d'une collection d'accessions de riz pour la tolérance au stress du froid au stade reproducteur. Cinquante cultivars de riz comprenant des génotypes exotiques et adaptés localement ont été évalués pendant deux saisons, soit les saisons

2015 A et 2016 B. Ces géotypes ont été plantés dans des conditions environnementales normales et plus tard exposés à 17°C pendant 30 jours. Les données ont été recueillies sur le nombre de tiges, la hauteur de la plante, le nombre de panicules, la saillie paniculaire et la fertilité des épillets, en utilisant des procédures standard. Les résultats ont révélé des différences significatives ($P < 0,001$) entre les géotypes. De plus, une relation significative ($r = 0,91$, $P < 0,001$) a été observée entre la saillie paniculaire et la fertilité des épillets, indicateurs de la tolérance au froid au stade de la reproduction. Les géotypes tolérants à basse température ont été identifiés. Cela nécessite des essais supplémentaires dans les régions montagneuses de l'Ouganda. Toutefois, les variétés tolérantes au froid identifiées sont à grains courts, mais les consommateurs de la région préfèrent le riz à grains longs. Ainsi, il est nécessaire d'incorporer la tolérance au froid dans les variétés préférées.

Mots clés: Accessions, tolérant au froid, germoplasme, *Oryza sativa*, Ouganda

Background

Rice (*Oryza sativa* L.) is a cereal crop and an important staple food for millions of people in many areas of the world (Ortiz, 2011). Rice can be grown in different environments depending upon water availability and temperature conditions. Rice is a cold-sensitive plant that originated from tropical or subtropical zones and the critical stages for cold damage include germination, booting, flowering, and grain filling stages. During the reproductive stages, low temperatures affect yield components causing overall yield loss (Changrong *et al.*, 2008). This is partly through delaying heading which results into pollen sterility (Suzuki *et al.*, 2008; Sudesh, 2010; Yadav, 2010). Various phenotypic symptoms in response to cold stress include poor germination, stunted seedlings, yellowing of leaves (chlorosis), reduced leaf expansion and wilting, and may lead to death of tissue (necrosis). There are currently no rice varieties that are tolerant to low temperatures in Uganda. Expanding area under rice production in the country by utilizing highlands would contribute towards meeting the food demand of the rapidly growing human population. This study aimed to identify cold tolerant genotypes for cultivation in highland regions.

Materials and Methods

The study was conducted in a screenhouse at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). MUARIK is located in Wakiso district, 25 km north of Kampala (32° 34'E, 0° 32'N) in Uganda at 1140m. a.s.l. It receives mean annual precipitation of 1200mm. Its average temperature per year is 23°C with annual minimum and maximum of 17°C and 33 °C, respectively. A total of 50 rice lines (accessed from Tanzania, Rwanda and Uganda) were screened to determine their level of tolerance to cold at reproductive stage (booting). Germplasm was arranged into two groups, one of them was kept at 17°C for 30 days while the other was left under uncontrolled lowland temperatures. The experiment was done twice from June 2015 to January 2016 also December 2015 to June 2016. Seeds were pre-germinated and after two weeks were transplanted into five litre plastic pots with two plants per pot. Pots were arranged in a

split plot design with temperature treatments as main plot and genotypes as subplot, with five replications. Plants were kept in the screenhouse and when two or more tillers in a pot reached reproductive stages (booting), that pot was taken to an artificially lighted room where the cold temperature treatment was initiated. However, due to the differences in varietal phenology, not all varieties were exposed to cold stress at the same time, i.e., cold treatment to various genotypes was staggered depending on their reproductive development time. The cold treatment room had a constant temperature of 17°C, and after 30 days of cold exposure, the pots were taken back to normal temperatures. At maturation, plants were evaluated for the degree of panicle exertion and spikelet fertility. Degree of panicle exertion was measured as the relative distance from the flag leaf ligule to the panicle ciliar node using a scale of 1- 9 (IRRI, 2014) where 1 means well exerted and 9 enclosed. Spikelet fertility was identified by pressing the spikelet with fingers and noting those that did not have grains. A scale of 1- 9 developed by IRRI (2014) was used to score fertility level where 1 was highly fertile (>90%) and 9 infertile (0%). Data were subjected to analysis of variance (ANOVA) using GenStat software (12th edition) at $P < 0.05$. The relationship between spikelet fertility and panicle exertion was established through regression analysis.

Results

Analysis of variance showed a significant season effect for all parameters evaluated except spikelet fertility. Significant temperature effect was observed for panicle exertion, tiller number and spikelet fertility. Panicle number and plant height were not significantly affected while the interaction effect of treatment and season was only significant for plant height and panicle exertion. Genotype, genotype by treatment, genotype by season and the interactions were highly significant for tiller number, panicle number, plant height, panicle exertion and spikelet fertility ($P < 0.001$) (Table 1).

Table 1. Mean squares for cold tolerance of rice at reproductive stage across two seasons.

SOV	DF	MS panicle number	MS plant height	MS tiller number	MS panicle exertion	MS spikelet. Fertility
Season	1	5416.44***	58154.4 ***	4953.83**	86.97**	0.67 ns
Treatment	1	23.77 ns	18448.1 ns	285.29**	1721.59***	6803.36*
Treatment.season	1	0.86 ns	11304.8 ***	15.37ns	130.48***	21.13 ns
Rep.seas	8	182.01	2406.7	309.92	3.923	4.271
MP error	8	40.39	312.3	18.56	1.932	7.844
Genotypes	49	84.93 **	1790.8***	55.01***	30.07**	30.41***
G*T	49	33.98 ***	302.1***	32.09***	12.13***	25.96***
G*S	49	40.85 ***	557.2***	26.3***	4.995***	2.66***
G*S*T	45	16.46 ***	480.5***	23.5***	2.76**	2.05***
SP-error	592-602	8.31	125.9	10.74	1.47	1.016

Ns = non-significant (**, ***) = significant at (0.01, 0.001) level respectively.

A strong positive correlation ($r = 0.91$, $P < 0.001$, $n = 50$) was detected between panicle exertion and spikelet fertility for both seasons (Table 2). Regression analysis revealed that panicle exertion explained 81% and 83% of variations in spikelet fertility in season one and two, respectively (Figure 1).

Table 2. Correlation between spikelet fertility and panicle exertion for 50 rice genotypes as influenced by cold temperature in two seasons

Parameters	Season	spikelet fertility		Panicle exertion	
		1	2	1	2
spikelet fertility	1	1			
	2	0.98***	1		
Panicle exertion	1	0.90***	0.91***	1	
	2	0.88***	0.91***	0.80***	1

*** = significant at $P < 0.001$

Genotypic differences were observed in rice as a result of exposure to cold temperatures. The results showed that genotypes which were cold tolerant were the short grain Japonica types. The Indica types were not tolerant to cold. Japonica rice cultivars are reported to have evolved in temperate regions and therefore have special adaptation to cold while Indica rice that was domesticated in the tropical climates are sensitive. At reproductive stage most Japonica types are reported to be tolerant to cold (Visperas and Vergara, 1981).

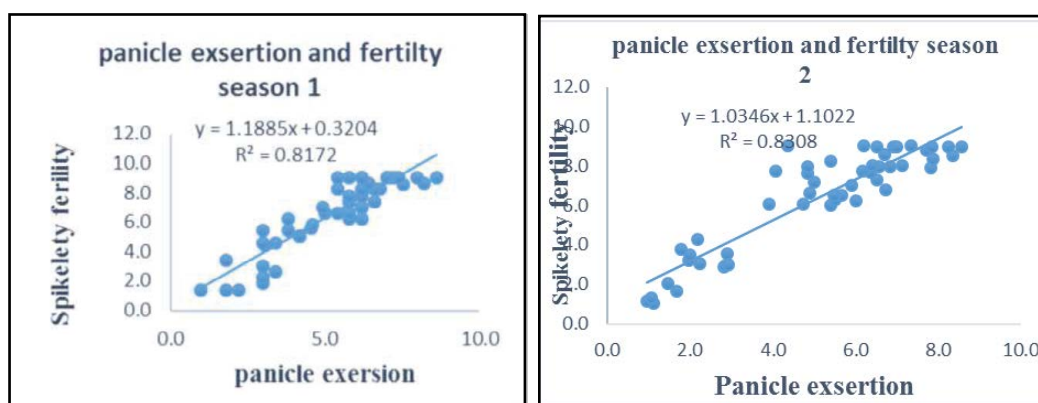


Figure 1. Regression analysis of spikelet fertility as affected by panicle exertion in season one (a) and season two (b)

Strong positive correlation between panicle exertion and spikelet fertility showed that selection can be done using one of the two traits ($r = 0.90$ and 0.88 , $R^2 = 0.82$ and 0.83 for season one and two, respectively) depending on the screening method. Cruz *et al.* (2008) noted that panicle exertion is a better cold tolerant indicator under field condition than

spikelet fertility (sterility) when they evaluated the F₂ generation in the field. Cold tolerance was measured as the percentage of reduction in panicle exertion and in spikelet fertility. According to Cruz *et al.* (2006) when cold tolerance was evaluated based on reduction in panicle exertion, it was not possible to distinguish between cold tolerant and cold sensitive genotypes. When the reduction in spikelet fertility was considered, a minimum of seven days was required to differentiate genotypes for cold tolerance. Panicle exertion technique could therefore be used for screening large populations while the spikelet fertility could be used for a small population.

Applications

The study revealed that there was a lot of genetic variability in terms of cold stress tolerance among the 37 exotic germplasm and 13 locally adapted varieties used in this study. Results showed that some genotypes were indeed tolerant to low temperature. These need to be tested in highland regions of Uganda. However, cold tolerant genotypes happened to be the short grained Japonica type that most people in the region do not like, preferring long grained rice types. This needs to be taken into account in developing germplasm for highland areas in Uganda.

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