GENOTYPIC RESPONSE IN RICE DURING THE REPRODUCTIVE PHASE UNDER WATER STRESS AND NON-WATER STRESS CONDITIONS

 M usila, R.N.^{1,2}, Sibiya, J.¹, Derera, J.¹, Kimani, J.M.² and Tongoona, P.¹

¹African Centre for Crop Improvement, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01 Scottsville 3209, Pietermaritzburg, South Africa ²Kenya Agricultural and Livestock Research Organisation, P. O. Box 57811-00200, Nairobi, Kenya

[Email: ruthmusila@gmail.com](mailto:Correspondence%20Email:%20ruthmusila@gmail.com)

ABSTRACT

Drought stress during the reproductive stage is a major constraint limiting rice production and productivity in rainfed upland and lowland ecologies especially in sub-Saharan Africa. This study was conducted to determine response of rice landraces and cultivated rice to water stress at reproductive growth stage, to identify sources of drought tolerance among selected rice landraces grown in coastal region of Kenya and to identity traits contributing to high grain yield under water stress conditions. Fifteen rice genotypes were evaluated in a randomized complete block design with four replications under water stress and non-water stress conditions in a steel and wire mesh screen house where weather conditions were uncontrolled. Data collection included canopy temperature, relative leaf water content, leaf rolling, and leaf drying, days to 50% flowering, spikelet fertility and grain yield per plant. The study revealed that there were no significant differences among rice genotypes for all the physiological traits measured under non-water stress conditions. However, under water stress conditions, genotypes varied significantly (P≤0.001) for all the physiological traits and in days to 50% flowering, spikelet fertility and grain yield per plant. The intensity of stress observed in this study was moderate as revealed by a relative yield reduction of 57%. Based on a selection index ranking, two local cultivars, *Shingo la Mjakazi* and *Kitumbo* were found to be moderately water stress tolerant and therefore potential sources of drought tolerance trait. All the other landraces were identified as water deficit susceptible. Under water stress conditions spikelet fertility showed a strong positive correlated with grain yield (0.62**) and was the most important contributor to higher grain yield and may be targeted to indirectly select for grain yield under water deficit conditions

Keywords: Drought tolerance, Landraces, Selection index, Spikelet fertility

INTRODUCTION

Rice production and productivity in sub-Saharan Africa (SSA) is limited partly by abiotic and biotic factors which vary significantly across growing environments and countries. Among the abiotic constraints, drought continues to prevail as the most important constraint limiting rice production and yield stability by smallholder farmers in rainfed upland and lowland ecologies in SSA (Seck *et al.*, 2010; Diagne *et al.*, 2013). The available cultural practices for drought mitigation during the early stages of rice growth and development usually result in a drop in the rice yields (Pandey *et al.*, 2007). When drought occurs late in the season, for example, during flowering or grain filling stage, flexibility in making management adjustment is limited resulting in drastic yield reduction and may even lead to total crop failures (Pandey *et al.*, 2007). In addition, most small-scale farmers growing rice in the rainfed ecologies are resource constrained and cannot afford small and minor irrigation facilities. Therefore, cultivation of drought tolerant cultivars may perhaps be the best option for rice drought management in SSA.

Approaches for development of drought resistant rice cultivars involve intensive screening of genotypes under drought conditions during either the vegetative (Efisue et al., 2009), reproductive or ripening phases (Anyaoha et al., 2018). The reproductive stage is the most sensitive to water stress and grain yield is reduced most when drought stress occurs during this stage (Rang *et al.*, 2011; He and Serraj, 2012). The strong effects of drought on grain yield are due to reduction of spikelet fertility and panicle exertion (Wassmann *et al.*, 2009). Methods developed to screen rice genotypes for drought resistance at reproductive stage range from managed field stress (Pantuwan *et al.*, 2002) to pot experiments (Wade *et al.*, 2000) under fully to semicontrolled conditions in greenhouses or in open fields.

The former allows mass screening, while the latter is suitable for pre-breeding work to evaluate specific germplasm, parental lines or mapping population. Pot experiments eliminate the confounding effects of heterogeneity of soil and moisture supply commonly associated with field screening. They increase the precision with which pure genotypic differences can be detected. In situations where the test materials differ in maturity period, timing of stress in relation to flowering date is of paramount importance and staggered planting is used to effectively synchronize

flowering during treatment period (Blum, 2011). In drought screening trials, a number of physiological and integrative traits have been identified as indicators of drought resistance at reproductive growth stage (Lafitte *et al.*, 2003). Some physiological traits recommended include relative water content, canopy temperature, leaf rolling and leaf drying scores (Lafitte *et al.*, 2003; Pantuwan *et al.*, 2002). Leaf rolling is the initial dehydration symptom observable when rice and other cereals are exposed to water stress. As plant water deficit progresses, leaf desiccation and death follow beginning with lower leaves and proceeds upwards. Relative leaf water content directly measures the actual water content of a leaf relative to its water content at full turgor (Blum, 2011; Mullan and Pietragalla, 2012).

Canopy temperature is an indirect measure of plant water status. In rice, infrared thermometry of leaf canopies has been found to be very effective for drought tolerance phenotyping (Ingram *et al.*, 1990). Among the integrative traits, spikelet fertility is the main yield component affected when stress occurs during the reproductive stage (Ekanayake *et al.*, 1989; Lafitte *et al.*, 2003). Although grain yield under stress is the primary trait for selection in breeding for drought prone environments (Lafitte *et al.,* 2003), the use of secondary traits together with yield in a selection index enhances selection efficiency (Blum, 2011).

Sources for drought resistance have been reported among wild, cultivated rice and landraces (Liu *et al.*, 2004; Zhang *et al.*, 2006). Agnihotri *et al.* (2009) observed that the rice landraces in Kumaun region of the Indian Central Himalaya had higher stomatal conductance, transpiration rate, water use efficiency and chlorophyll content in comparison to an introduced variety VL-206. Within the cultivated Asian rice, Liu *et al.* (2004), reported that some cultivated rice that included Azucena and WAB 56-50 possessed alleles for improved root growth and distribution under water deficit. In a study involving 325 BC2F2 bulk populations, developed by backcrossing drought tolerance donors to elite recurrent parents, Lafitte *et al.* (2006), reported presence of cryptic genetic variation for drought tolerance even in the drought-susceptible cultivars. The cultivated African rice has long been identified as a source of drought resistance among other traits (Zhang *et al.*, 2006; Olembo *et al.*, 2010). It has, therefore, been utilised in interspecific crossings with the Asian rice producing another source of drought resistance within the NERICA cultivars (Lamo, 2009; Olembo *et al.*, 2010). This study evaluated popular landraces and local cultivars in the coast region of Kenya, and few selected exotic materials from the African-Rice Centre (ARC), the International Centre for Tropical Agriculture (CIAT) and the International Rice Research Institute (IRRI) in order to: (i) determine response of rice landraces and introduced genotypes to water stress at reproductive growth stage, (ii) to identify superior genotypes using a selection index, iii) to identity traits contributing to high grain yield under water stress conditions.

MATERIALS AND METHODS Study Location

The study was conducted on-station at Kenya Agricultural and Livestock Research Organization (KALRO)-Mtwapa. KALRO-Mtwapa is located 20 km north of Mombasa in Kilifi County, along Mombasa-Malindi road. It lies on latitude 3°50'S and longitude 39°44'E at an elevation of 15 m above sea level (m asl). Annual mean temperatures range between $22^{\circ}C$ and 26^oC. The area receives bimodal rainfall of about 1200 mm with reliable long rains of 600 mm falling around mid-March to July and variable short rains of 250 mm falling around mid-October to December. The soils are dominated by orthic acrisols (80% sand) with low inherent fertility (Jaetzold and Schmidt, 1983). The typical agro-ecological zonation for KARLO-Mtwapa is coastal lowland three (CL3-coconut cassava zone).

Germplasm

The germplasm consisted of 15 rice genotypes, which had not been previously evaluated for tolerance to water stress under local environment. The source and characteristics of the genotypes are given in Table 1.

Experimental design and crop management

The 15 rice genotypes were evaluated under water stress and non-water stress conditions from November 2013 to March 2014. The experimental materials were sheltered in a steel and wire mesh screen house. The roof of the screen house was covered with a clear polythene paper to shelter the materials from rainfall. Sides were open having only wire gauze while the floor was covered with a clear polythene paper to prevent roots imbibing water from the soil. There was free flow of air inside the screenhouse thus light, carbon dioxide concentration and temperature were uncontrolled. Soil for planting was upland soil which was first sieved (2mm) and sterilized by dry heating in a hot air oven at 80°C for 2 hours and left to cool. The sterilized soil was mixed with sand and coconut coir dust in the ratio of 2:1:1, respectively. Seeds of the selected genotypes were germinated in the sterilized soil mixture. Fifteen days' later, seedlings were transplanted in black polyethylene pots of 25 cm internal diameter and 30 cm height filled with 18 kg of the soil mixture. Before transplanting, the pots were watered with equal amount of water to raise the soil moisture to 100% water holding capacity. The experimental design was randomized complete block design replicated four

times. The plot size was ten pots per entry. In each pot four seedlings were transplanted and spaced at 10 cm each to give a total of 40 plants per plot. During planting, diamonium phosphate (DAP) was applied as a source of P. The P was applied at recommended rate of 60 kg P ha⁻¹ each pot receiving 0.48 g of P Source of N was calcium ammonium nitrate (CAN) which was top dressed at the rate of $120 \text{ kg N} \text{ ha}^{-1}$ applied in three splits of 40 kg ha $^{-1}$ (0.32 g per pot) at 21 days after transplanting, tillering stage and at panicle initiation stage. Weeds were controlled by hand picking. Harvesting was carried out manually.

Table 1. Source, type and some characteristics of the 15 medium to late maturing genotypes used in the study

Genotype	Source*	Species	Characteristics
Kitumbo	Kenya	Oryza sativa	Landrace, late, poor grain quality
Tuliani	Kenya	Oryza sativa	Landrace, late, good grain quality and highly aromatic
Supaa	Kenya	Oryza sativa	Landrace, late, good grain quality and highly aromatic
Kibawa Chekundu	Kenya	Oryza sativa	Landrace, late, good for confectionery purposes
Shingo la Mjakazi	Kenya	Oryza sativa	Landrace, medium to late, good for confectionery purposes
Basmati 370	Kenya	Oryza sativa	Landrace, medium, highly aromatic
Nerica L-19	ARC	Interspecific	Medium, long slender grains
Nerica L-25	ARC	Interspecific	Medium, long slender grains
Luyin 46	IRRI	Oryza sativa	Medium, high yielding, high tillering
IR10LL151	IRRI	Oryza sativa	Medium, high tillering
IR10LL176	IRRI	Oryza sativa	Medium, high tillering
FKR19	ARC	Oryza sativa	Medium, good gain quality, high tillering
IR74371-54-1-1	IRRI	Oryza sativa	Medium, high reproductive stage drought tolerance
IR55423-01	IRRI	Oryza sativa	Medium, moderate reproductive stage drought tolerance,
Azucena	IRRI	Oryza sativa	Medium to late, reproductive stage drought susceptible

Drought screening

The genotypes were divided into two maturity groups in order to synchronize flowering. The medium maturing (115 to 120 days) group was planted 20 days later after the late maturing group (140 to 150 days) Up to 70 days after sowing (based on medium maturity lines) each pot received one and half litres of water on a daily basis. At the beginning of drought treatment, soil moisture in all pots was zero centibars. The soil water content was monitored using watermark sensors installed in two pots per replication. Using a watermark meter model 200SS-5 designed to read watermark sensors exclusively, readings were monitored on daily basis and the average computed. Two consecutive drying cycles of water stress were imposed (Figure 1).

Figure 1. Soil moisture tension in centibars during the two cycles of drought screening

In the first cycle, soil moisture tension increased from 0 to 79 centibars by the eighth day. Most plants had started showing symptoms of wilting. On the $9th$ and 10th day of water stress, soil moisture in all pots was raised to 100% water holding capacity. The second cycle of water stress was imposed on the $11th$ day. During the second cycle, soil moisture tension increased from 0 to 81 centibars by the seventh day. Thereafter, the soil moisture tension was maintained at between 30 to 40 centibars until harvesting. The nonwater stress experiment received the same cultural practices as the stress treatment except that each pot received one and half liters of water on daily basis until grain filling stage. Thereafter the water was reduced to one and half liters after every two days maintain the soil moisture at 100% until plants were ready for harvesting as recommended by Bimpong et al., 2011.

Data collection

Measurements of the drought related physiological characters namely, canopy temperature (CT) in ${}^{\circ}C$, relative leaf water content (RLWC) in %, leaf rolling (LR) score and leaf drying score were taken during the water stress period at the panicle initiation. The Standard Evaluation System (SES) for rice reference manual (IRRI, 1996), was used for all trait measurements except where stated otherwise. Measurements were taken as observed on the whole plot basis. Canopy temperature was measured using infrared thermometer. Measurements we recorded from 11 to 13 h when there was little or no wind. Two measurements were taken and the mean was computed. Relative leaf water content was determined between 12 and 14 h by the method suggested by Barrs and Weatherley (1962). From each plot 2-3 leaf samples mid leaf-section of about 5-10 cm were cut with scissors. Each sample was placed with its basal part to the bottom, in a pre-weighed airtight oven proof vial slightly longer than the samples. Vials were placed in a cooler box (10-15°C) and transported to the laboratory immediately. In the lab, vials were weighed to obtain leaf sample fresh weight (FW). After weighing deionized water was added to each vial and samples were left to hydrate for 24 hours under normal room light and temperature. After hydration samples were taken out of water, dried and immediately weighed to obtain fully turgid weight (TW). The samples were oven dried at 80°C for 72 hours and weighed after cooling in a desiccator to determine the dry weight (DW).

Relative leaf water content was calculated as: RLWC = {(Fresh weight - Dry weight)/ (Turgid weight - Dry weight)} x 100. Leaf rolling was scored on a scale of 0 to 9: where $0 =$ healthy leaves; 1= shallow V shaped leaves; $3 =$ deep V-shaped leaves; $5 =$ fully capped, Ushaped leaves; $7 =$ leaf margins touching (0-shape); $9 =$ tightly rolled leaves (IRRI 1996). During the period of drought imposition three scores were taken per plot and averaged. Leaf drying was scored at the end of the stress period. A scale of 1 to 5 was used where 1 indicated no of leaf death whereas 5 corresponded to complete plant death. Three scores were taken per plot. Days to 50% flowering (DFL) was determined visually when the central tiller of half of the selected had anthers exerted. Delay in flowering determined by subtracting days to 50% flowering under drought conditions from days to 50% flowering under no drought conditions. Spikelet fertility was determined as described by Lafitte *et al.* (2003). Twenty panicles were randomly selected from each plot. Spikelet fertility was scored as; highly fertile (>90%); fertile (75-89%); partly sterile (50-74%); highly sterile \langle <50% to trace) and completely sterile (0%). Grain yield per plant was determined on whole plot basis. The grain was harvested manually, hand threshed, and the grains dried to achieve a moisture content of 14%. The grain was weighed using a digital electronic balance. The mean grain weight obtained from the ten plants was computed to give grain yield per plant in grams.

Statistical data analysis

The analysis of variance (ANOVA) was performed according to (Gomez and Gomez, 1984) to determine differences among treatments and genotypes for each variable using GenStat statistical package version 14 (Payne *et al.*, 2011). The treatment and genotype means were separated using the least significant differences (LSD) test. To determine levels of drought tolerance of each genotype, a selection index as suggested by (Bänziger *et al.*, 2000) was used to summarise the worth of each genotype as follows; Weights (Wi) were assigned based on the relative value of each trait as an indicator of drought stress in upland rice ecology. The phenotypic values, Pi, were standardized, as: $Pi = (xii - mi)/sdi$; where *mi* and *sdi* are the mean and standard deviation of trait *i* in the experiment, and *xij* is the value of the trait *i* measured on genotype *j*. A selection index SI for each genotype was then computed as: $SI = W1PI + W2P2 + ...$ *WnPn* where *Pi* is the observed standardized value of the trait *i* and *Wi* is the weight assigned to that trait in the selection index (Bänziger *et al.*, 2000). These weights were determined based the correlation of the trait with grain yield and ease of measurement and repeatability of each trait in the field. Weights assigned for selected traits were; canopy temperature 3, relative leaf water content 1, leaf rolling 3, spikelet fertility 4 and grain yield per plant 5. The checks were used for rating drought tolerance and susceptibility of the other genotypes. Simple linear correlation analysis was also computed to determine association between the studied traits using GenStat statistical package version 14

(Payne *et al.*, 2011). The relative yield reduction between the stress and non-water stress (RYR%) was estimated according to Kumar et al., 2008 as RYR% = $100 \times [1 - (Grain yields a *Stress* / (Grain yields a *Stress*)$

RESULTS AND DISCUSSION

Analysis of variance

The results of combined analysis of variance indicated mean squares due to genotypes were highly significant $(P < 0.01)$ for all traits indicating that the genotypes performed differently under stress and non-stress conditions. Highly significant genotype x environment interaction was observed for all traits except for canopy temperature. Variation in physiological response to water stress at reproductive stage among rice genotypes has been reported for leaf rolling and death (Pantuwan *et al.*, 2002; Kumar *et al.*, 2014), canopy temperature (Garrity and O'Toole 1995) and for relative leaf water content (Bimpong *et al.*, 2011; Kumar *et al.*, 2014).

Table 2. Combined analysis of variance for physiological traits, grain yield, spikelet fertility and days to 50% flowering among 15 rice genotypes evaluated under water stress and non-stress conditions at Mtwapa Kenya

Source of variation		Mean squares										
		CT†	RLWC	i R	LD	DFL	SF	$GYP-1$				
ENV		$621.08**$	$26669.82**$	832.13**	$165.68**$	$1548.01**$	$31145.03**$	6235.33**				
REP(ENV)		5.55	48.98	0.16	0.16	27.74	130.25	72.47				
GENOTYPE	4	9.88**	150.56**	$5.40**$	$00**$	2316.39**	$332.60**$	$286.95**$				
ENV*GENOTYPE	14	$6.45*$	$164.67**$	$5.40**$	$00**$	$20.58**$	198.23**	56.07**				
RESIDUAL	84	2.83	28	0.71	0.08	7.28	39.07	23.23				
CV		6.34	7.31	23.18	13.05	2.74	8.91	26.74				

*: $P < 0.05$ and **: $P < 0.01$

† CT, Canopy temperature; DFL, Days to 50% flowering; GYP-1 , Grain yield per plant; RLWC, Relative Leaf Water Content; LR, Leaf rolling; LD, Leaf drying; SF, Spikelet fertility.

Effect of water stress on genotypes

There were significant variations among traits under non-stress and stress conditions. The relative yield reduction under water stress varied among genotypes recording an average of 57% above control (Table 3). Yield reduction was mild in the highly drought tolerant check - IR74371-54-1 (31%) and severe in genotypes NERICA-L-25 and FKR19 each with yield reduction of 79%. The genotypes IR10LL176 and Luyin 46 consistently showed high yields under stress and nonstress conditions and hence it can be said that yield potential was an indicator of their performance under water stress conditions. The intensity of stress observed in this study was moderate and similar to that observed in other studies under water stress at reproductive stage (Kumar *et al.*, 2009; Verulkar *et al.*, 2010).

Spikelet fertility is the main yield component affected when stress occurs during the reproductive stage because it leads to irreversible processes of yield reduction (Ekanayake *et al.*, 1989; Lafitte *et al.*, 2003). Water stress reduced spikelet fertility of all the genotypes. Reduction in spikelet fertility varied from 26 to 55% with an average of 37% over control. The landrace *Kitumbo* and IR74371-54-1, showed the lowest spikelet fertility reduction of 26%. Basmati 370 and IR10LL151 recorded the highest spikelet fertility reduction of 55 and 54%, respectively. High spikelet sterility resulted from retention of mature spikelets inside the flag leaf sheath prohibiting the opening of spikelets. White and discoloured empty spikelet's were observed in genotypes such as Bas370, NERICA-L-25 and FKR19, indicating that these genotypes were highly drought sensitive. Flowering delay is an expression of drought susceptibility in the affected genotype. Water stress delayed days to 50% flowering ranging from zero to 14 days with a mean of eight days. Similar delays in flowering have been reported by other researchers Anyaoha et al., 2018. Delayed flowering was not observed in the highly drought tolerant check -IR74371-54-1, confirming that this genotype had high reproductive stage drought tolerance. The genotype NERICA-L-25 had the longest delay of 15 days indicating that it was drought susceptible. The delay in flowering observed may have been predisposed by higher canopy temperature and low relative water content. Delayed flowering is a strong indicator of susceptibility to drought because of retarded growth.

Stress increased canopy temperature of all the genotypes with a mean canopy temperature increase of 19% above the control (Table 3). Canopy temperature of *Kitumbo,* a landrace was the least affected by stress at 6% increase indicating that this genotype may be drought tolerant. NERICA-L-25 experienced the highest increase in canopy temperature of 36% which may have been predisposed by high stomatal closure and low transpiration rate under drought stress hence highly drought sensitive. Canopy temperature was an indirect measure of internal water status and important predictor of yield performance under drought.

Genotype	Non water stress				Water stress										
	GYP †	SF	DFL	CT	RLWC	LR	LD	GYP	SF	DFL	CT	RLWC	LR	LD	RYR
	g	$\frac{0}{0}$	days	$\rm ^{o}C$	$\frac{0}{0}$	Score	Score	g	$\frac{0}{0}$	days	$\rm ^{o}C$	$\frac{0}{0}$	Score	Score	$\frac{0}{0}$
IR74371-54-1-1	30.55	95.59	78	23	86.00	1.00	1.00	21.04	70.39	78	26	68.49	2.50	2.00	31
Luyin 46	31.65	89.02	85	24	87.00	1.00	1.00	19.94	63.82	91	28	56.26	6.00	3.50	37
Nerica L-19	29.72	89.19	89	24	87.50	1.00	1.00	15.94	60.16	97	27	54.73	7.00	3.00	46
Shingo la Mjakazi	19.53	91.60	95	25	85.50	1.00	1.00	10.30	54.86	97.5	27	69.60	4.00	2.25	47
Kitumbo	17.29	73.93	121	26	87.50	1.00	1.00	8.95	54.38	126	27	53.38	5.50	3.50	48
IR10LL176	37.04	90.19	89	24	87.50	1.00	1.00	18.28	60.11	96	28	60.43	6.00	3.25	51
IR55423-01	34.45	91.75	88	24	88.50	1.00	1.00	15.76	63.83	90	27	66.67	4.25	2.25	54
Supaa	16.39	73.53	121	25	89.25	1.00	1.00	7.06	51.79	132	30	56.75	7.00	4.00	57
Kibawa Chekundu	13.32	82.38	117	25	88.50	1.00	1.00	5.03	50.89	127	32	54.14	8.50	4.00	62
Azucena	19.08	90.88	94	24	87.50	1.00	1.00	6.93	52.80	102	29	66.87	7.50	3.75	64
Tuliani	21.62	74.12	118	25	89.50	1.00	1.00	6.88	52.03	131	31	52.98	7.50	4.00	68
Bas 370	21.81	85.57	84	25	85.75	1.00	1.00	6.55	38.49	96	30	44.67	8.00	4.00	70
IR10LL151	30.08	89.01	83	24	86.75	1.00	1.00	7.79	40.57	90	29	65.02	6.00	3.75	74
FKR19	30.98	95.42	80	23	85.50	1.00	1.00	6.56	44.78	94	28	53.67	7.00	3.00	79
Nerica L-25	25.02	81.60	85	24	87.75	1.00	1.00	5.29	51.56	99	32	39.10	7.25	4.00	79
Mean	25.24	86.25	95	24	87.33	1.00	1.00	10.82	53.36	103	29	57.52	6.27	3.32	57
LSD(0.05)	6.33	6.84	4	$\mathbf{2}$	3.11	0.00	0.00	7.38	10.48	$\overline{\mathbf{4}}$	3	10.22	1.70	0.57	

Table 3. Means of measured traits under non-stress and reproductive drought stress conditions at KALRO Mtwapa, Kenya

† CT, Canopy temperature; DFL, Days to 50% flowering; GYP-1 , Grain yield per plant; RLWC, Relative Leaf Water Content; LR, Leaf rolling; LD, Leaf drying; RYR, Relative Yield Reduction; SF, Spikelet fertility.

Garrity and O'Toole (1995), found this trait to be very effective for field screening for drought avoidance phenotyping in rice. The relative leaf water content (RLWC) estimates the volumetric water content of the leaf tissue relative to its capacity at full turgidity and could be regarded as a measure of water deficit in the plant leaf (Blum, 2011). The leaf water content reduction due to water stress varied from 19 to 55% with an average relative reduction of 34% over control. Other researchers have also reported similar levels in their studies (Bimpong *et al.*, 2011; Kumar *et al.*, 2014). Reduction in leaf water content was more pronounced for NERICA-L-25 at 55%.

The pronounced drought effects recorded for NERICA-L-25 may have been caused by the warmer canopy temperatures observed in this genotype under stress conditions. *Shingo la Mjakazi* (19%) and the drought tolerant check, IR74371-54-1-1 (20%) showed the lowest reduction in leaf water content indicating that these genotypes were less affected by drought compared to the other genotypes and therefore, possibly drought tolerant. Leaf rolling is a wellrecognized dehydration symptom extensively used by breeders in selecting for avoidance of water stress in rice (O'Toole and Cruz, 1980; Blum, 2011). In this study, leaf rolling of all the genotypes was affected by

water stress ranging from deep V shaped (score of 3) to tightly rolled leaves (score of 9). Likewise, all the genotypes showed signs of leaf drying from slight (score of 2) to severe (score of 4). The drought tolerant check showed the lowest leaf rolling and leaf drying scores of 3 and 2, respectively confirming its potential to tolerate water stress at reproductive growth stage. *Kibawa chekundu* performed poorly with leaf rolling and drying scores of 9 and 4, respectively. It was observed that leaf rolling and death were more pronounced among genotypes that showed higher percentages of increased canopy temperature and pronounced reduction in relative leaf water content. These genotypes were also larger in plant size which may have resulted in more transpiration demand predisposing the genotypes to more water stress.

Selection index

The selection index (SI) values ranged from -6.38 to 7.78 and negative values were more desirable and indicated drought tolerance (Table 4). The highly drought tolerant check - IR74371-54-1-1 was exceptional with a SI of -6.38 contributed by a good combination for increased yields and spikelet fertility and decreased canopy temperature and leaf rolling under water stress conditions.

Table 3. Ranking of the 15 rice genotypes based on selection index

Table of Kanking of the 10 Title genotypes based on selection much										
Genotype	CT^*	RLWC	LR	SF	$GYP-1$	SI	Ranking			
IR74371-54-1-1	-3.27	1.02	-5.97	0.48	1.37	-6.38				
Shingo la Mjakazi	-1.75	1.13	-3.6	0.37	0.67	-3.18	2			
Kitumbo	-1.91	-0.39	-1.22	0.37	0.58	-2.56	3			
IR55423-01	-1.66	0.85	-3.2	0.43	1.02	-2.55	$\overline{4}$			
Nerica L-19	-2.86	-0.26	1.16	0.41	1.03	-0.52	5			
Luvin 46	-0.88	-0.12	-0.43	0.43	1.29	0.3	6			
IR10LL151	-0.36	0.7	-0.43	0.27	0.51	0.69	7			
FKR19	-0.61	-0.36	1.16	0.30	0.43	0.92	8			
IR10LL176	-0.51	0.27	-0.43	0.41	1.19	0.93	9			
Supaa	1.41	-0.07	1.16	0.35	0.46	3.31	10			
Azucena	-0.01	0.87	1.95	0.36	0.45	3.62	11			
Bas370	1.62	-1.2	2.74	0.26	0.43	3.85	12			
Nerica L-25	4.2	-1.72	1.55	0.28	0.34	4.66	13			
Tuliani	2.59	-0.42	1.95	0.35	0.45	4.91	14			
Kibawa Chekundu	3.89	-0.32	3.53	0.34	0.33	7.78	15			

† CT, Canopy temperature; DFL, Days to 50% flowering; GYP-1 , Grain yield per plant; RLWC, Relative Leaf Water Content; LR, Leaf rolling; LD, Leaf drying; SF, Spikelet fertility.

Shingo la Mjakazi a landrace was ranked second and showed a good combination of decreased canopy temperature and leaf rolling and increased leaf water content. *Shingo la Mjakazi* and *Kitumbo* were ranked higher than the moderately drought tolerant check and may be classified as moderately drought tolerant. The lowest ranking genotype was *Kibawa Chekundu* and showed highest positive SI values (7.78) contributed by increased canopy temperature and leaf rolling, and decreased spikelet fertility and grain yield. Based on the index none of the improved varieties outperformed the moderately drought tolerant check - IR55423-01. While the landraces, Supaa, BAS370, Azucena and Tuliani and Kibawa chekundu showed low values for

yield and were ranked lowest indicating their susceptibility to drought stress. Based on yield and selection index Anyaoha et al., 2018, also reported that most unimproved varieties ranked lowest compared to improved varieties showing their susceptibility to reproductive-stage drought stress.

Correlation among characters

Correlation among characters was investigated under both water stress and non-stress conditions, but only the results on association of characters under stress conditions are presented in Table 5. Under non-stress conditions significant and positive association was observed between grain yield per plant and spikelet fertility (0.55***) and negative association between grain yield and days to 50% flowering (-0.59***). The rest of the traits did not show any significant association with grain yield per plant. Under water stress conditions grain yield had a significant positive correlation with RLWC (0.35**) and significant negative association with all the drought related traits. This is in agreement with other studies that reported that relative leaf water content, canopy temperature, leaf rolling and drying scores were correlated to higher yield or yield stability under drought stress (Lafitte *et al.*, 2003; Pantuwan *et al.*, 2002). Further, significant and positive association was observed between grain yield per plant and spikelet fertility (0.62***). A simple linear regression revealed that spikelet fertility was the most important factor contributing to higher grain yield per plant under water stress conditions (Table 6). These findings are similar to those revealed by other researchers (Lafitte *et al.*, 2003; Zou *et al.*, 2005) and suggest increased spikelet fertility indirectly contributes to higher grain yield under water deficit conditions during the reproductive stage. Thus, the best approach to indirectly select for increased grain yield is to select for higher spikelet fertility.

Table 4. Phenotypic Pearson correlation coefficients between grain yield plant-1 and physiological traits, days to 50% flowering and spikelet fertility under drought conditions

to bo /0 nowering and spincier ierancy under drought conditions										
Plant characteristics	$GYP-1$	CT	RLWC	LR	LD	DFL	SF			
Grain yield per plant (GYP^{-1})	X									
Canopy temperature (CT)	$-0.43**$	X								
Relative leaf water content (RLWC)	$0.35**$	$-0.35**$	X							
Leaf rolling (LR)	$-0.50***$	$0.58***$	$-0.38**$							
Leaf drying (LD)	$-0.48***$	$0.55***$	$-0.43**$	$0.68***$	Х					
Days to 50% flowering (DFL)	$-0.39**$	$0.37**$	-0.18 ns	$0.45***$	$0.43**$	X				
Percent spikelet fertility (SF)	$0.62***$	$-0.42**$	$0.30*$	$-0.40**$	$-0.44***$	$-0.31*$	X			

*, **, ***, Significant at p< 0.05, 0.01 and 0.001 probability levels, respectively; ns, non-significant

CONCLUSIONS

Subjecting rice genotypes to water stress exposes much useful variation that may not be obvious under optimum conditions and this allows a breeder to select for potential drought tolerant genotypes. In this study genotypes performed differently in response to water stress and two landraces *Kitumbo* and *Shingo la Mjakazi* were less affected by water stress since they showed minimum reduction in canopy temperature, reduced delay in 50% flowering and decreased spikelet infertility compared to other landraces. In addition, the selection index ranked *Shingo la Mjakazi* and *Kitumbo* higher than the moderately drought tolerant check - IR55423-01 indicating that they may possess moderate tolerance to water deficit at reproductive stage. Thus these two landraces could be used in breeding aimed at developing drought tolerant improved rice varieties. This study also observed that spikelet fertility was the most important factor contributing to higher grain yield per plant under water stress conditions. Breeders may use spikelet fertility in combination with the other physiological traits to indirectly select for grain yield under water stress conditions.

ACKNOWLEDGEMENTS

The authors have not declared any conflict of interests. We are grateful to the Alliance for a Green Revolution in Africa (AGRA) for providing funds for this study through the African Centre for Crop Improvement (ACCI), University of KwaZulu Natal, Republic of South Africa. The authors thank Kenya Agricultural and Livestock Research Organization (KALRO) management for granting study leave to the first author.

REFERENCES

- Agnihotri, R.K., Palni, L.M.S., Chandra, S. and Joshi, S.C. 2009. Gas exchange variability and water use efficiency of thirty landraces of rice still under cultivation in Kumaun region of the Indian Central Himalaya. Physiology and Molecular Biology of Plants, 15:303-310.
- Anyaoha, C.O., Fofana, M., Gracen, V.E., Tongoona, P.B., Blay, E.T., Semon, M. and Popoola, B. 2018. Yield potential of upland rice varieties under reproductive-stage drought and optimal water regimes in Nigeria. Plant Genetic Resources: Characterization and Utilization, 16(4):378–385.
- Bänziger, M., Edmeades, G., Beck, D. and Bellon, M. 2000. Breeding for drought and nitrogen stress tolerance in maize: From theory to practice. CIMMYT, Mexico, D.F.
- Barrs, H.D. and Weatherley, P.E. 1962. A reexamination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences, 15(3):413-428.
- Bimpong, I.K., Serraj, R., Chin, J.H., Mendoza, E.M.T., Hernandez, J. and Mendioro, M.S. 2011. Determination of genetic variability for physiological traits related to drought tolerance in African rice (*Oryza glaberrima*). Journal of Plant Breeding and Crop Science, 3(4):60-67.
- Blum, A. 2011. Plant Breeding for Water-Limited Environments. Springer, New York, USA. p. 53- 55.
- Diagne, A., D.Y. Alia, E., Amovin-Assagba, M.C.S Wopereis, K. Saito and T. Nakelse. 2013. Farmer perceptions of the biophysical constraints to rice production in sub-Saharan Africa, and potential impact of research, p. 46-68. In: Wopereis, M.C.S. et al. (Eds.). *Realizing Africa's Rice Promise*. CAB International.
- Efisue, A., Tongoona, P., Derera, J. and Ubi, B. 2009. Screening early-generation progenies of interspecific rice genotypes for drought-stress tolerance during vegetative phase. Journal of Crop Improvement, 23(2):174-193.
- Ekanayake, I., Datta, S.K.D. and Steponkus, P. 1989. Spikelet sterility and flowering response of rice to water stress at anthesis. Annals of Botany, 63(2):257.
- Fukai, S. and Cooper, M. 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. Field Crops Research, 40(2):67-86.
- Garrity, D.P and O'Toole, J.C. 1995. Selection for reproductive stage drought avoidance in rice, using infrared thermometry. Agronomy Journal, 87(4):773-779.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research*,* 2nd ed. John Wiley and Sons, New York, USA.
- He, H. and Serraj, R.. 2012. Involvement of peduncle elongation, anther dehiscence and spikelet sterility in upland rice response to reproductive-stage drought stress. Environmental and Experimental Botany, 75:120-127.
- Ingram, K.T., Real, J.G., Maguling, M.A., Obien, M.A. and Loresto, G.C. 1990. Comparison of selection indices to screen lowland rice for drought resistance. Euphytica, 48(3):253-260.
- IRRI. 1996. Standard Evaluation System for Rice. International Rice Research Institute, Los Bańos, Philippines
- Kumar, A., Verulkar, S., Dixit, S., Chauhan, B., Bernier, J., Venuprasad, R., Zhao, D. and Shrivastava, M. 2009. Yield and yield-attributing traits of rice (*Oryza sativa* L.) under lowland drought and suitability of early vigor as a selection criterion. Field Crops Research, 114:99-107.
- Kumar, S., Dwivedi, S.K., Singh, S.S., Jha, S.K., lekshmy, S., Elanchezhian, R., Singh, O.N. and Bhatt, B.P. 2014. Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. Journal of Breeding and Genetics, 46(2):217-230.
- Lafitte, H.R., Li, Z.K.C., Vijayakumar, H.M., Gao, Y.M., Shi, Y., Xu, J.L., Fu, B.Y. and Yu S.B. 2006. Improvement of rice drought tolerance through backcross breeding: evaluation of donors and selection in drought nurseries. Field Crops Research, 97:77-86.
- Lafitte, R., Blum, A. and Courtois, G. 2003. Secondary traits to help identify drought-tolerant genotypes. In: K. S. Fischer (ed.). Breeding rice for droughtprone environments. International Rice Research Centre (IRRI), Los Bańos, Philippines. p. 37-48.
- Lamo, J. 2009. Genetic studies on drought tolerance and grain shattering in rice. Ph.D thesis, University of KwaZulu-Natal, Republic of South Africa.
- Liu, L., Lafitte, R. and Guan. D. 2004. Wild *Oryza* species as potential sources of drought-adaptive traits. Euphytica, 138:149-161.
- Olembo, N., M'mboyi, F. and Oyugi, K. 2010. Success stories in crop improvement in Africa: The case of rice in sub-Saharan Africa. African Biotechnology Stakeholders Forum (ABSF), Nairobi, Kenya.
- O'Toole, J.C. and Cruz, R.T. 1980. Response of leaf water potential, stomatal resistance, and leaf rolling to water stress. Plant Physiology, 65:428.
- Pandey, S., Bhandari, H., Ding, S., Prapertchob, P., Sharan, R., Naik, D., Taunk, S.K. and Sastri, A. 2007. Coping with drought in rice farming in Asia: Insights from a cross country comparative study. Agricultural Economics, 37:213-224.
- Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S. and O'Toole, J.C. 2002. Yield response of rice (*Oryza sativa* L.) genotypes to drought under rainfed lowland: 3. Plant factors contributing to drought resistance. Field Crops Research, 73:181- 200.
- Payne, R.W., Murray, D.A. Harding, S.A. Baird, D.B. and Soutar. D.M. 2011. GenStat for Windows (14th Edition) Introduction. VSN International, Hemel Hempstead.
- Rang, Z.W., Jagadish, S.V.K., Zhou, Q.M., Craufurd, P.Q. and Heuer, S. 2011. Effect of high temperature and water stress on pollen germination and spikelet fertility in rice. Environmental and Experimental Botany, 70(1):58-65.
- Seck, P.A., Tollens, E., Wopereis, M.C.S., Diagne, A. and Bamba, I. 2010. Rising trends and variability of rice prices: Threats and opportunities for sub-Saharan Africa. Food Policy, 35(5):403-411.
- Verulkar, S., Mandal, N., Dwivedi, J., Singh, B., Sinha, P., Mahato, R., Dongre, P., Singh, O., Bose, L. and Swain, P. 2010. Breeding resilient and productive genotypes adapted to drought-prone rainfed ecosystem of India. Field Crops Research, 117:197-208.
- Wade, L.J., Kamoshita, A., Yamauchi, A. and Azhiri-Sigari, T. 2000. Genotypic variation in response of rainfed lowland rice to drought and rewatering. Plant Production Science, 3:173-179.
- Wassmann, R., Jagadish, S., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R., Howell, G., Pathak, H. and Sumfleth. K. 2009. Climate Change Affecting Rice Production:: The Physiological and Agronomic Basis for Possible Adaptation Strategies. Advances in Agronomy, 101:59-122.
- Zhang, X., S. Zhou, Y. Fu, Z. Su, X. Wang and C. Sun. 2006. Identification of a drought tolerant introgression line derived from dongxiang common wild rice (*O. rufipogon* Griff.). Plant Molecular Biology, 62:247-259.
- Zou, G.H., Mei, H.W., Liu, H.Y., Liu, G.L., Hu, S.P., Yu, X.Q., Li, M.S., Wu, J.H. and Luo, L.J. 2005. Grain yield responses to moisture regimes in a rice population: Association among traits and genetic markers. Theoretical and Applied Genetics 112(1):106-111