Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage

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ABSTRACT

Food shortage is a major problem in sub Saharan Africa as population increases. Over 89% of Kenyans are food poor and are malnourished especially in rural areas. NERICA (New Rice for Africa) are high yielding rainfed rice varieties with early maturity and has shown high potential to revolutionize rice farming even in Africa's stress afflicted ecologies. However, NERICA varieties vary in their response to water deficit. A pot experiment was conducted in 2009 at the Maseno University Botanic garden, to evaluate the responses of five NERICA varieties (NERICA1, NERICA 2, NERICA 3, NERICA 4 and NERICA 5) to water deficit during their vegetative or reproductive stage of their development. The response pattern of physiological and biochemical parameters to water deficit imposed at different growth stages might provide basis for selecting the most tolerant variety to water deficit in order to stabilize yield and solve food crisis. The treatments were; T_1-irrigating the pots with a litre of water after every two days (Control), T_2-water deficit at vegetative stage in which water was withheld by irrigating the plants using one litre of water after every six days from 30-50 days after planting; T_3-water deficit at reproductive stage in which water was withheld by irrigating the plants using one litre of water after every six days from 51-71 days after planting. Water deficit caused a significant reduction in physiological parameters i.e. growth, chlorophyll fluorescence and biochemical parameters i.e. chlorophyll and protein content both at vegetative stage and at reproductive stage. However, Growth parameters i.e. plant height, root length and plant dry weight were affected more by water deficit imposed at the vegetative stage as compared to water deficit imposed at the reproductive stage while chlorophyll fluorescence, chlorophyll content and protein content were affected more by water deficit imposed at reproductive stage as compared to water deficit at reproductive stage. The results indicate that NERICA 2 and 4 were tolerant as compared to NERICA 1, 3 and 5 to water deficit occurring at vegetative stage or reproductive stage because their growth, chlorophyll fluorescence and biochemical parameters were least affected.

Key words: NERICA rice, Relative water content, Plant biomass, Chlorophyll fluorescence, Chlorophyll and protein content

INRODUCTION

Majority of the population in developing nations including Kenya is characterized by a high population of the poor households whose entire livelihood depends on farming as an economic activity and drought has worsened the poverty status, but some rice varieties can perform well in areas with limited rainfall. Rice is the staple food for more than half of the world's population and is now a commodity of strategic significance driven by changing food preference in the urban and rural areas and compounded by increased urbanization (Khalil *et al.,* 2009). In many countries rice accounts for more than 70% of human caloric intake. Most Kenyans living in the rural areas consume limited quantities of rice although it forms an important diet for the majority of urban dwellers (MOA, 2008). Rice yields have been increasing since the 1960's but since the 1990's growth in rice production has been slower than population growth (Mwaura, 2010). It is anticipated that rice production will need to increase by 30% by 2025 in order to sustain those who need it for sustenance. However, climate change especially access to water threaten rice yields. Water deficit has been described as the single physiological and ecological factor upon which plant growth and development depends more heavily than other factors (Kramer and Boyer, 1995). Any shortage in water supply in relation to the requirement of plants

results in water deficit hence plants become stressed. Water deficit evoke responses in plants which are based on the development of physiological drought making soil water unavailable to the plant. Water content has been widely used to quantify the water deficits in leaf tissues. Leaf water content is a useful indicator of plant water balance since it expresses the relative amount of water present on the plant tissues (Yamasaki and Dillenburg, 1999). The species adapted better to dry environments have higher relative water content at given water potential. Water deficit also causes leaf water potential and rates of elongation to decline more rapidly in rice than in maize or sorghum so that dry matter accumulation and nutrient uptake decline or cease. Leaf dehydration can be minimized by decreasing evapotranspiration or by increasing water absorption from the drying soil (Chaves et al., 2003). It has been established that water deficit is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Anjum et al., 2003). Water stress causes deceleration of cell enlargement and thus reduces stem lengths by inhibiting inter nodal elongation and also checks the tillering capacity of plants. The importance of root system in acquiring water has long been recognized. A prolific root system can confer the advantage to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation in legumes (Johansen et al., 1992). Differences in root length could confer tolerance to drought by some varieties. Greater plant fresh and dry weights under water deficit conditions are desirable characters. A common adverse effect of water deficit on crop plants is the reduction in fresh and dry biomass production. However some genotypes shows better stress tolerance than the others. Studies by Mohammadian et al. (2005) showed that mild water stress affected the shoot dry weight while shoot dry weight was greater than root dry weight loss under severe stress in sugar beet genotypes. Wullschleger et al. (2005) reported a decrease in the root dry weight under mild and severe water stress in populous species. Chlorophyll is one the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate. Chen et al.(2007) noted that assessment of pigment content has become an effective means of monitoring plant growth and estimating photosynthetic productivity while Fillella et al.(1995) reported that remote estimates of pigment concentration provides

an improved evaluation of the spatial and temporal dynamics of plant stress. Chlorophyll concentration has been known as an index for evaluation of source therefore a decrease of this can be consideration of a non stomata limiting factor in the drought stress conditions. Water stress is reported to inhibit the incorporation of amino acids into proteins and to cause a decrease in the protein content of the tissues. Water deficit impedes protein synthesis at the ribosomal level: some proteins are apparently formed and inactivated quickly whereas others appear to be relatively stable. Studies on sunflower by Rao et al. (1987) showed that water deficit reduces seed protein content. Protein content, particularly soluble protein usually falls to about 40-60% of the initial content as the water deficit becomes intense in drought sensitive plants. Leaf chlorophyll fluorescence probe is a powerful and sensitive intrinsic measurement of the photosynthetic process that can be used to detect the influence of various environmental stress factors. According to Maxwell and Johnson (2000) the measurement of chlorophyll fluorescence in situ is a useful tool to evaluate the tolerance of the photosynthetic apparatus to environmental stress which reduces the maximum efficiency of PSII photochemistry. It is used to determine how light use efficiency for photosynthesis occurs at the cellular level. It can also be used to estimate the activity of the thermal energy dissipation in photosystem II which protects photosystems from the adverse effects of light and heat stress.

The objective of this study was to evaluate the response pattern of growth, chlorophyll fluorescence and biochemical parameters of five NERICA (NERICA1, NERICA 2, NERICA 3, NERICA 4 and NERICA 5) varieties to water deficit occurring at vegetative stage or reproductive stage of their development which might provide basis for selecting the most tolerant variety to water deficit in order to stabilize yield and solve food crisis.

MATERIALS AND METHODS

The study was carried out at the Maseno University Botanic Garden under green house conditions between June 2008 and Jan 2010. The green house was naturally illuminated and the light, CO_2 concentration and temperature conditions were not controlled. Conditions during the study were; day temperature ranged from 22 - 34^{0}C, relative humidity from 50 – 90% and photon flux density (PPFD) from 400 – 600 µmol photons $m^{-2}s^{-1}$. Seeds of five New Rice for Africa (NERICA) rainfed rice varieties namely

NERICA 1, 2, 3, 4 and 5 coded as N-1, N-2, N-3, N-4 and N-5 were obtained from the NERICA adaptability trials in the Maseno University Botanic garden. The soil was dug from the garden, solarized for one week then filled into 20 litre plastic pots up to ¾ full. The soils at Maseno are classified as acrisol being well drained, deep clay with pH ranging between 4.6 and 5.4 (Sikuku et al., 2010). The seeds were soaked in water at 30^0C for 72 hours prior to planting to facilitate germination. The pots were laid out in a Randomized Complete Block Design (RCBD). The seeds were sown at the rate of four seeds per hill and there were 4 hills per pot with a spacing of 15 x 25 cm and planting depth of 3cm. The treatment combinations consisted of three levels of water regimes, viz. T_1 - well watered throughout the life cycle in which the plants were watered with one litre of water after every two days throughout the growing period, T_2 - water deficit at vegetative stage in which water was withheld by irrigating the plants using one litre of water after every six days from 30-50 days after planting, T_3 - water deficit at reproductive stage in which water was withheld by irrigating the plants using one litre of water after every six days from 51-71 days after planting. One litre of water was used to irrigate all the pots after every two days for 28 days to maintain optimum moisture before initiating experimental treatments. Plants were irrigated after every six days from 30 to 50 days after planting to impose water deficit at vegetative stage and from 51 to 71 days after planting to impose water deficit at reproductive stage. After water deficit period, plants were irrigated after every two days. Three replications were performed for each treatment and each variety. The experiment was repeated twice.

Relative leaf water content: Relative leaf water content was determined on the flag leaf of twelve plants per treatment for all replications at 28, 42, 56, 70 and 84 days after sowing. The leaves to be harvested were rinsed with distilled water to eliminate surface accumulation of salts two hours before harvesting. The sampled leaves were cut at the base of the lamina and one gram of each weighed immediately to get the fresh weight (W_f). The leaf disks were then placed in a test tube containing distilled water for 24 hours at room temperature to get the turgid weight (W_t). The disks were dried in an oven at 80^oC until a constant weight was obtained to get the oven dry weight (W_d). The relative water content was calculated using the formula of Coombs et al. (1985) as follows: Relative water content (R) = $(W_f – W_d)/ (W_t - W_d) \times 100$

Plant height: Shoot height was determined on twelve hills per treatment and per replication at 28, 42, 56, 70 and 84 days after sowing. Measurements were made using a metre rule from the stem base to shoot apex in plants.

Root length: At the end of the experiment, the plants were uprooted, soil particles were washed off the roots and roots blotted dry using paper towels. The length of the roots was then determined using a meter rule from the stem base to the longest root tip of the main root.

Plant biomass

Whole plant fresh and dry weights At the end of the experimental period, whole plants per hill were uprooted; the harvested plants were partitioned into roots, shoots and leaves. The roots were washed in tap water to remove the soil particles and blotted dry on paper towels. The fresh weights were determined immediately after harvesting using an electronic weighing balance (Denver instrument model XL - 31000). The samples were placed in paper bags and oven dried at 80^0C for 4 days to a constant dry weight. The dry weights of the samples were then determined and the data used to determine root to shoot ratio.

Chlorophyll content determination: Two methods were used to determine leaf chlorophyll content i.e. destructive and non destructive measurements.

(i)Destructive Measurements: Chlorophyll was extracted on the flag leaf of twelve plants per treatment and per replication at 28, 42, 56, 70 and 84 days after sowing using methods of Arnon (1949) and Coombs et al. (1985). One gram of the harvested fresh leaf tissue was weighed and cut into small pieces and placed into a specimen bottle containing 10 ml of absolute ethanol then stored in the dark for two weeks. 1 ml of the filtered extract was then diluted with 6 ml of absolute ethanol and the absorbance read against ethanol blank using a spectrophotometer (Ultrospec 11) at 645 and 663nm to determine chlorophyll a (chl.a) and chlorophyll b (chl. b) content of the leaf tissue. Chlorophyll a and b content in milligrams of chlorophyll per gram of leaf tissue was calculated according to Arnon (1949) using the following formula:

Mg chl.a /g leaf tissue = 12.7(D663)-2.67(D645) x V/1000 x W

Mg chl.b /g leaf tissue = 22.9(D645)-4.68(D643) x V/1000 x W

Where D = Absorbance at wavelengths 645 nm and 663 nm

V = Volume (ml) of the ethanol extract

W = Fresh weight of leaf tissue

(ii) Non destructive measurements: Chlorophyll content (SPAD Index) of flag leaf of twelve plants per treatment and per replication was estimated non destructively using a portable chlorophyll meter (SPAD-502 Minolta Co. Japan). This index was used preferentially because the strong relationship between readings of portable chlorophyll meter and leaf chlorophyll content has been demonstrated by several authors (Silva *et al.,* 2007; Markwell *et al.*, 1995). The measurements were carried out between 0930 and 1300 hrs at 28, 42, 56, 70 and 84 days after sowing.

Protein content determination: Protein was extracted from the flag leaf of twelve plants per treatment and per replication at 28, 42, 56, 70 and 84 days after sowing. In the laboratory, one gram of the harvested fresh leaf tissue was weighed and cut into small pieces into specimen bottles, mixed with 10 ml of 2% anhydrous Sodium Carbonate in 0.1 M NaOH and the set- up stored for one month for the protein to be extracted. 0.5 ml protein suspension extracted was mixed with 0.5 ml of a reagent containing 48 ml of 2% anhydrous Na_2CO_3 in 0.1 M NaOH, 1 ml of 0.5% $CuSO_4$, and 1ml of 1% sodium potassium tartate. The solution was allowed to stand for 15 minutes after which 0.5 ml of Folin-Ciocalteau reagent was added and the solution left to stand for a further 30 minutes at room temperature. The absorbance of the protein solution was measured using a spectrophotometer (Ultrospec 11) at 700 nm. The Protein content was estimated by the Lowry method as described by Coombs *et al.* (1985) using bovine serum albumin as a standard.

Chlorophyll fluorescence measurements: Chlorophyll fluorescence measurements were taken at 28, 42, 56, 70 and 84 days after sowing on intact upper unfolded leaf of twelve plants per treatment for all replications with a portable fluorescence monitoring system (Hansatech model FMS 2; Hansatech Instruments, Germany).Twelve plants per treatment and per replication were sampled and the measurements done between 0930 and 1300 hrs. Prior to fluorescence measurements, a circular surface of the upper face of the leaves were dark adapted for 15 minutes using the dark adaptation clips. An actinic pulse light was used to saturate the photosystems. The initial fluorescence (Fo) and the maximum fluorescence level (Fm) was measured.

The variable fluorescence (Fv) was calculated as (Fv = Fm – Fo) and the maximal quantum yield of PSII photochemistry (Fv/Fm) was determined (Belkhodja *et al.*, 1999). The electron transport rate (ETR) and quantum efficiency of photosystem II (PSII) were also measured.

Statistical analysis of data: Analysis of variance (ANOVA) was carried out on the data for the variables measured during the study period to test for differences between the treatments and the varieties using a statistical computer package (SAS). The treatment and variety means were separated using the least significant differences (LSD) test at 5% level.

RESULTS

Relative water content

Leaf Relative water content: There was a highly significant interaction ($P \leq 0.05$) among the varieties, treatments and DAS. Water deficit caused a significant reduction in leaf water content and the highest reduction among the varieties was at water deficit treatment during reproductive stage as compared to vegetative stage. NERICA 2 and 4 recorded higher leaf water content as compared to NERICA 1, 3 and 5 at water deficit treatments during vegetative (Fig.1a) and reproductive stage (Fig.1b).
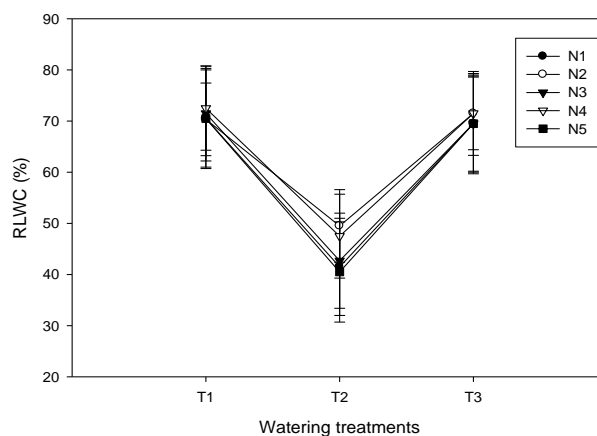

Fig.1a. Relative leaf water content (%) at DAS 42 (Vegetative stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ±SE). LSD (0.05) =0.4385 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)
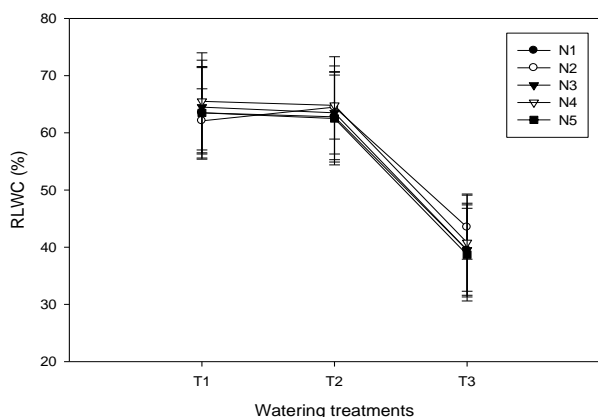
Fig.1b. Relative leaf water content (%) at DAS 70 (Reproductive stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) =0.4385. (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Plant height: Plant height was significantly (P ≤ 0.05) reduced by water deficit treatment both at vegetative and reproductive stage of plant growth. Subjecting the plants to water deficit at the vegetative stage caused a significant decrease in height as compared to water deficit imposed at the reproductive stage. The interaction between the varieties and treatments was significant (P ≤ 0.05). Varieties had a significant effect (P ≤ 0.05) in plant height at water deficit treatments. N-1 registered the highest height at the well watered treatment while N-2 had the highest height at water deficit treatments (Fig. 2).
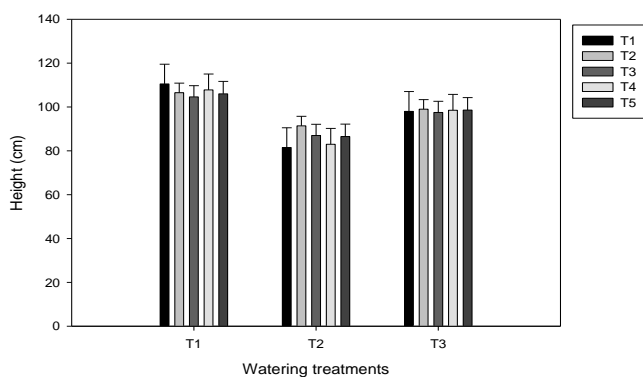

Fig.2. Height (cm) at Maturity of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ±SE). LSD (0.05) =0.4106. (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Root length: There was a significant (P ≤ 0.05) effect among the treatments and among the varieties in root length. Water deficit generally caused a reduction in root length with plants exposed to water deficit during vegetative stage being the most affected. N-4 had the highest length at control and at water deficit treatments while N-5 recorded the lowest length under water deficit treatments (Figure 3). Subjecting the varieties to water deficit at vegetative and reproductive stages caused decrease in root length relative to the control with NERICA 2 and 4 recording the lowest percentage reduction in root length both at vegetative and reproductive stage. However NERICA 1 and 5 were the most affected by water deficit both at vegetative and reproductive stage and had the highest percentage reduction from the control.
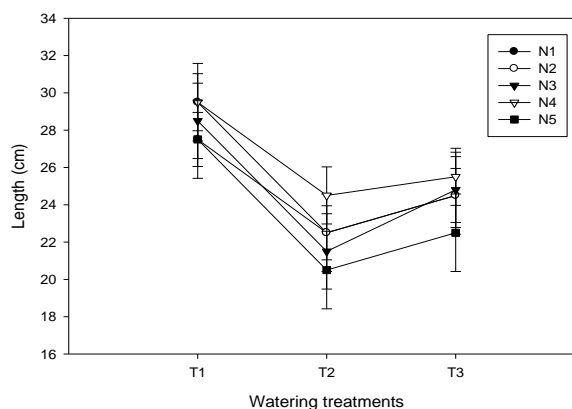

Fig. 3. Root length of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 1.3177 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Whole Plant dry weight: Analysis of variance results indicate that water deficit had a significant (P ≤ 0.05) inhibitory effect on plant dry weights of all the five NERICA varieties. Differences among the treatments were highly significant (P ≤ 0.05). However, the varietal difference was not significant (P > 0.05).The varieties were most affected by water deficit imposed at the vegetative stage (Fig. 4). NERICA 1 and 5 were the most affected by water deficit imposition recording the highest reduction from the control at water deficit treatment at vegetative and reproductive stages while NERICA 2 and 4 had the least reduction from the control.
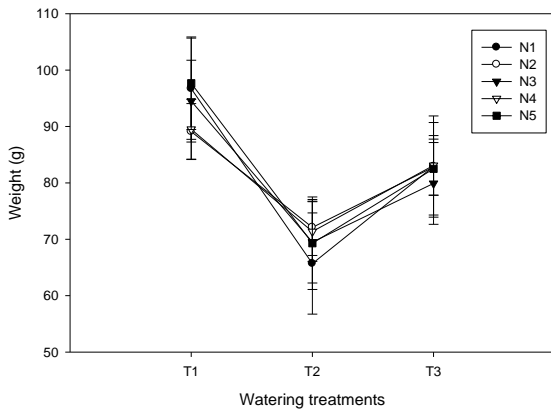
Fig. 4. Whole plant dry weight of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 1.9254 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Chlorophyll content: Chlorophyll a, b and total chlorophyll (SPAD INDEX) were significantly different (P ≤ 0.05) among the treatments and among the varieties. The variety x treatment x DAS had a highly significant interaction (P ≤ 0.05). NERICA 2 and 4 had the highest Chlorophyll content at the water deficit treatment at vegetative stage (Fig.5a) and reproductive stage (Fig.5b). N-1 was the most affected by water deficit imposition at the reproductive stage recording remarkably lower values relative to the control. However, there was no significant interaction (P > 0.05) between the varieties and treatments.
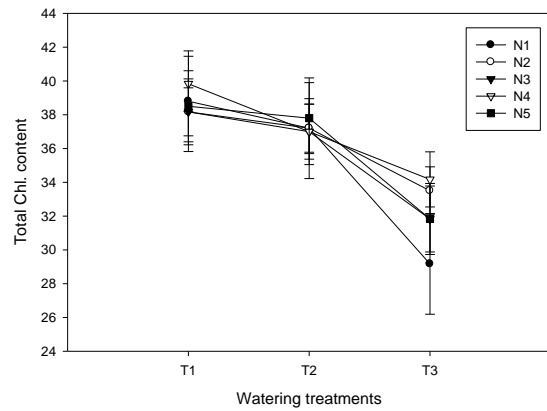

Fig. 5b. Total chlorophyll content (SPAD) at DAS 70 (Reproductive stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 0.4568 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Protein content: Protein content of leaves was significantly (P ≤ 0.05) reduced by water deficit imposition at the vegetative and reproductive stage. There was a significant effect (P ≤ 0.05) in protein content among the varieties with N-1 being tolerant to water deficit by showing higher protein content both at the control and during water deficit at the vegetative and reproductive stage (Figure 6a and b). N-4 had a remarkable reduction in protein content during water deficit at the vegetative stage relative to the control while N-3 was the most affected by water deficit at the reproductive stage.
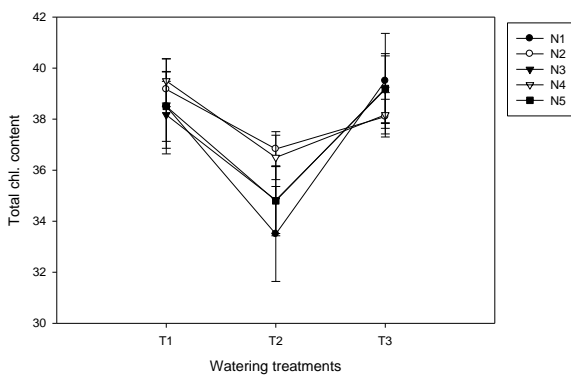

Fig. 5a. Total chlorophyll content (SPAD) at DAS 42 (Vegetative stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 0.4568. (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)
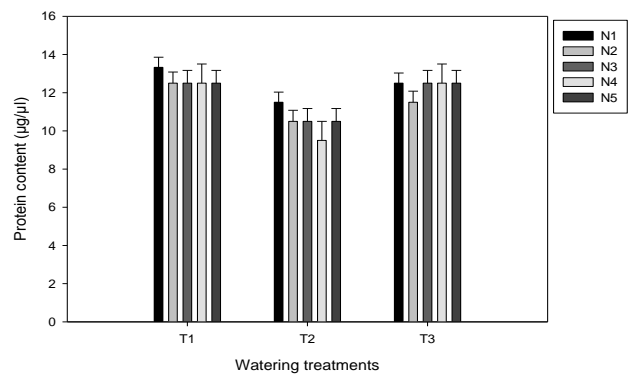

Fig. 6a. Leaves protein content at DAS 42 (Vegetative stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 0.3433 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)
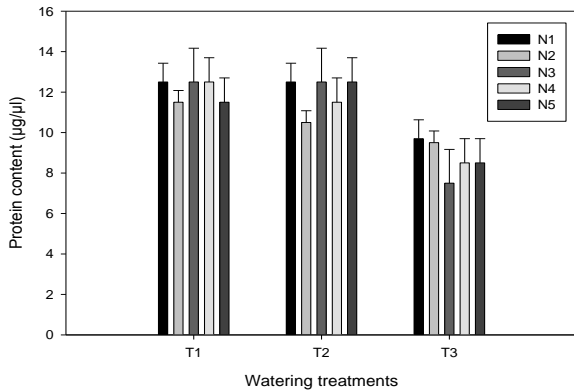
Fig. 6b. Leaves protein content at DAS 70 (Reproductive stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 0.3433 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Chlorophyll fluorescence: The Fv/Fm ratio which characterizes the maximal quantum yield of the primary photochemical reactions in dark adapted leaves was remarkably reduced by water deficit. The treatments differed significantly (P ≤ 0.05) and lower Fv/Fm values were recorded at water deficit treatment during reproductive as compared to vegetative stage. The varieties differed significantly (P ≤ 0.05) at water deficit treatments, N-2 had the highest chlorophyll fluorescence values during vegetative stage followed by N-4 (Fig.7a) while N-1 recorded the least Fv/ Fm ratio. At reproductive stage NERICA 2 and 4 had higher values as compared to NERICA 1, 3 and 5 as shown by Figure 7b.
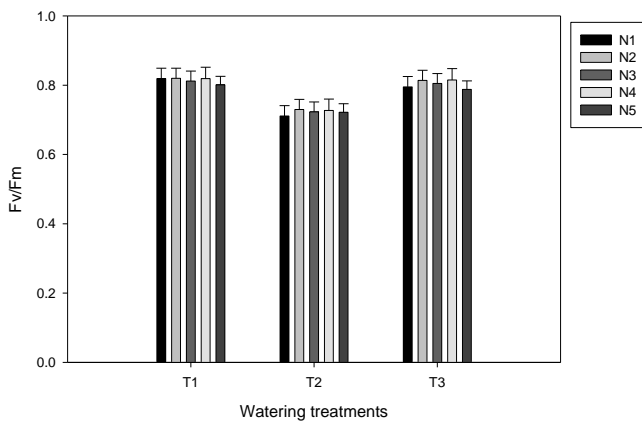

Fig.7a. Chlorophyll fluorescence at DAS 42 (Vegetative stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ±SE). LSD (0.05) =0.001 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)
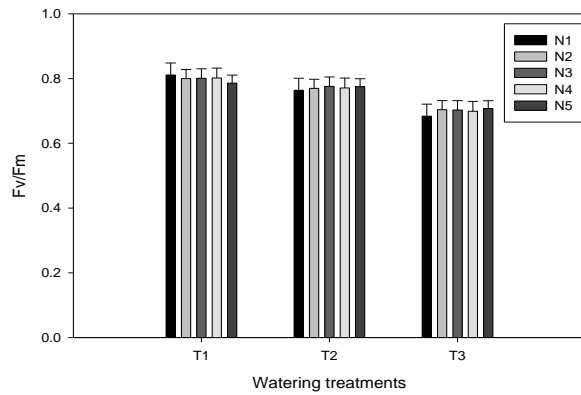

Fig.7b. Chlorophyll fluorescence at DAS 70 (Reproductive stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ±SE). LSD (0.05) =0.001 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Electron transport Rate: Electron transport rate exhibited significant effect (P ≤ 0.05) between treatments and among the varieties. The treatment effect was significant (P ≤ 0.05) with water deficit having the most negative effect on electron transport rate at reproductive stage as shown by Table 8b. The varieties differed significantly (P ≤ 0.05) in electron transport rate. At water deficit treatment during vegetative stage, N-2 had slightly higher ETR rates compared to the other varieties while N-1 was the most affected (Fig.8a). At the reproductive stage, NERICA 2, 3 and 4 had higher ETR values at water deficit treatment as compared to NERICA 1 and 5. However, there was no major difference in ETR rates among the varieties in treatment 1.
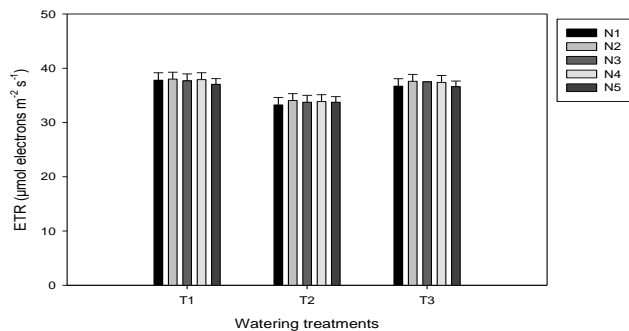

Fig.8a . Electron transport rate (µ mol electrons m^{-2} s^{-1}) at DAS 42 (Vegetative stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) = 0.039 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)

Fig.8b. Electron transport rate (μ mol electrons m^{-2} s^{-1}) at DAS 70 (Reproductive stage) of five NERICA rice varieties grown at three levels of watering treatments (Means of three replicates ± SE). LSD (0.05) =0.039 (T1- Well watered control, T2- water deficit at vegetative, T3- water deficit at reproductive)
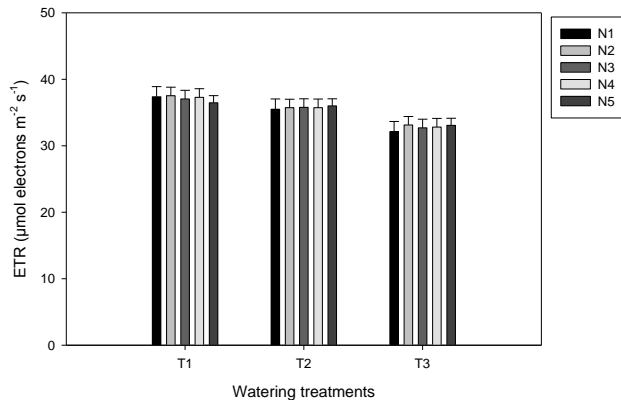
DISCUSSION

Water deficit at vegetative stage and reproductive stage caused a significant reduction in leaf water content (Fig. 1a and b). This result confirms previous work on durum wheat by Melkiche and Larbi (1992), showing the effects of water deficit on relative water content in wheat plants. Decrease in leaf water content may have been caused by water loss through evapotranspiration and decreased water absorption by the roots when the soil water was limiting (Chaves et al., 2003). Low relative water content which is developed due to water deficit has been reported to decrease the leaf growth rate and leaf area development, inhibits dry matter accumulation and accelerate leaf senescence, decrease photosynthesis, increase frequency of zygotic abortion, decrease grain number, size and yield (Sikuku et al., 2010). In the present study, low relative water content due to water deficit inhibited growth and plant function which were reflected in lower shoot dry matter, decreased chlorophyll content and lower chlorophyll fluorescence. The varietal difference was significant but only at water deficit treatments. N-2 was able to maintain the highest water content at reproductive stage and it had the lowest percentage reduction from the control (25%) while N-5 had the highest reduction (39%). The higher relative water content recorded by NERICA 2 and 4 at water deficit treatments may be attributed to their ability to absorb more water from the soil and the ability to control water loss through stomata. The varieties that are tolerant to drought have more

relative water content and relative water content can be used to select high yielding genotypes that maintain cell turgor under water deficit environments and give relative high yields. Changes in the relative water content of leaves are considered as a sensitive indicator of drought stress and more useful integrator of plant water balance than the leaf water potential. Plants subjected to water deficit at the vegetative stage showed relative water content values as high as control plants at reproductive stage suggesting that rewatering after the release of stress at the vegetative stage enabled full recovery of plant vigor. By the time plants attained the reproductive stage, the effects of water deficit imposed at the preceding growth stage had diminished. Siddique et al. (2000) reported a similar phenomenon in water stressed wheat. There was relative water content reductions of between 25-39%, this showed that some NERICA rice plants can maintain relatively high relative values despite the development of moisture stress. Similar results have been reported in Bambara groundnut (Vurayai et al., 2011). This is a very important trait which indicates drought tolerance as varieties which exhibit restricted changes in relative water content per unit reduction of water potential are often considered to be relatively drought tolerant (Vurayai et al., 2011). The tolerant variety which is able to maintain higher relative water content at moisture deficit would possibly maintain protoplast hydration for a longer duration under water deficit stress conditions thus ensure productivity.

Plant height was significantly reduced by water deficit treatment both at vegetative and reproductive stage. This finding is in agreement with the results of Nielsen and Nelson (1998), who reported depression of plant height as a result of water deficit. The decreased shoot growth may constitute an adaptive response to water deficit and may be attributed to the reduction in plant cell turgor which affected cell division and expansion. However, cell division has been reported to be less sensitive to water deficit than cell expansion or enlargement. The depression of plant height could also have resulted from a reduction in plant photosynthetic efficiency as reported by Castonguay and Markhart (1992). Water deficit at vegetative stage significantly reduced the plant height as compared to well watered treatment whereas water deficit at reproductive stage did not influence the plant height significantly as compared to well watered treatment (Fig. 2). Water deficit did not affect plant height much during reproductive stage because the plants had ceased growing vegetatively by this time. After rewatering the plants stressed

during the vegetative stage increased in plant height. This may be attributed to resumption of stem cell division and elongation plus leaf expansion. The varietal difference was significant. NERICA 1 and 4 were generally tall varieties and had the highest length at well watered treatment. This can be attributed to the inherent characteristic of the plants. At water deficit treatment during reproductive stage, N-2 had the least reduction (14%) from the control while N-1 had the highest reduction (25%). NERICA 2 demonstrated drought tolerance due to less reduction in height than NERICA 1, 3, 4 and 5 under drought conditions. This may have resulted from NERICA 2's ability to maintain relatively higher relative water content hence cell turgor at moisture deficit treatments. As noted by Salisbury and Ross (1992), cell enlargement requires turgor to extend the cell wall and a gradient in water potential to bring water in the enlarging cell but water deficit suppresses cell expansion and cell growth due to low turgor pressure.

Water deficit at vegetative and reproductive caused a reduction in root length. The decline in root length in response to drought might be due to either decrease in cell elongation resulting from the effect of water shortage on growth promoting hormones which in turn led to a decrease in cell turgor, cell volume and eventually cell growth. Water deficit caused more reduction in root length at vegetative stage compared to reproductive stage (Fig. 3). This shows that extensive root growth occurs at the vegetative stage hence water deficit at reproductive stage had little impact on root growth. In the present study, NERICA 2 and 4 had higher root lengths compared to NERICA 1, 3 and 5 at water deficit treatment during vegetative stage. NERICA 2 and 4 also recorded the lowest percentage reduction from the control both at water deficit at vegetative and at reproductive stage while NERICA 1 and 5 had the highest reduction. This implies that NERICA 2 and 4 were able to maintain root growth at water deficit treatment and apparently this characteristic contributed highly towards higher drought tolerance where deeper and extensive root systems contributed positively to water uptake (Huang and Gao, 1999).

Whole plant dry weight significantly declined with moisture deficit. This finding is in agreement with the results reported by Emmam et al. (2010). Water deficit may have influenced the height and leaf area per plant which ultimately influenced the shoot dry matter of plants. A reduction of photosynthetic surface by water deficit decreases the ability of plants

to produce dry matter. Moreover, the decline in both dry weight of shoots and roots of plants reveals the influence of water in stimulating and regulating the photosynthetic enzymes and growth promoting hormones which thus influences dry matter production. Water enhances cell division and promotes secondary wall formation. More recent studies have however shown that stem and leaf growth may be inhibited at low soil moisture content despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment. This suggests that the growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development of transpiring leaf area in the water stressed plants.   Lowest plant biomass values were recorded at plants exposed to water deficit at vegetative stage compared to reproductive stage and control (Fig. 4). The varietal difference was not significant, however, NERICA 2 and 4 had higher plant biomass at water deficit treatments and had the least reduction (19% and 20% respectively) from the control compared to NERICA 1, 3 and 5 (Fig.8b). The high total biomass accumulated by NERICA 2 and 4 may be as a result of the high root length which penetrated deep into the soil and absorbed water at levels where NERICA 1, 3 and 5 could not reach (Jones, 1996) and a generally lower transpiration rate.

Water deficit caused a reduction in Chlorophyll a, b and estimated total Chlorophyll (SPAD INDEX). Similar results have been observed in maize (Anjum et al., 2011) and barley (Kuroda et al., 1990). The decrease in chl.a may have been caused by the inhibition of biosynthesis of precursors of chlorophyll under moisture deficit as reported by Makhmudov (1983). The significant decrease in total chlorophyll content might be attributed to the increased degradation of chlorophyll pigments due to stress induced metabolic imbalance. Besides the inhibitory effect of decreased water content on leaf development, reduced stomatal conductance leading to decrease in carbon assimilation might have contributed to decreased chlorophyll content which ultimately affected transfer of photosynthetic assimilates from source to sink. The losses in chloroplast activity include decreases in the electron transport and photophosphorylation and may be associated with changes in conformation of the thylakoids and of coupling factor (ATP-synthatase- a sub unit of the thylakoids) and decreased substrate binding by coupling factor. In the five NERICA varieties under study, the most reduction in chlorophyll content occurred at water deficit treatment

during reproductive stage as compared to vegetative stage. These results implies that water deficit at reproductive stage produced more reactive oxygen species such as oxygen and hydrogen peroxidide which led to lipid peroxidation and consequently chlorophyll destruction (Sikuku *et al.*, 2010). The production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus; this might be avoided by degrading the absorbing pigments. Generally NERICA 2 and 4 had higher Chl. content compared to NERICA 1, 3 and 5 at water deficit treatments (Fig.5a and 5b). The results show that NERICA 2 and 4 are more suitable for rainfed areas because under water deficit conditions they were capable of maintaining high relative water content and their chlorophyll content had tendency to endure moisture deficit. Pastori and Trippi (1992) reported that tolerant varieties of wheat and corn had higher chlorophyll content than sensitive varieties under water deficit. Chen *et al.* (2007) noted that assessment of pigment content has become an effective means of monitoring plant growth and estimating photosynthetic productivity.

Protein content was significantly reduced by imposition of water deficit (Fig.6a and 6b). Similar results have been reported in maize (Kaiser, 1987). The decrease in protein content may have been due to the reduction in total protein synthesis and a rapid dissociation of polyribosomes. Protein synthesis may be particularly sensitive to water deficit for example most polysomes revert to monosomes under water deficit conditions. Water deficit is reported to inhibit the incorporation of amino acids into proteins and to cause a decrease in the protein content of the tissues. According to Jones *et al.* (1993), reduced protein synthesis appears to stem partly from diminished RNA synthesis and partly from a four fold increase in RNase activity because water enhances the synthesis of one of the alpha – amylase isozymes. Reproductive stage water deficit caused more reduction in protein content as compared to vegetative stage. This may imply that water deficit subjected at reproductive stage has more effect on plant's protein content as compared to water deficit subjected at the vegetative stage. N-1 showed tolerance to water deficit by having higher protein content at water deficit treatment as compared to NERICA 2, 3, 4 and 5.

The Fv/Fm ratio which characterizes the maximal quantum yield of primary photochemical reactions in leaves was significantly reduced by water deficit at vegetative and reproductive stage. The patterns of changes in fluorescence parameters observed in this study are consistent with the pattern of change reported under water deficit conditions by Araus *et al.* (1993). This suggests that electron transport from PSII to PSI in NERICA was adversely affected by water deficit. The decrease in Fv/ Fm may be attributed to the down regulation of photosystem II activity and impairment of photochemical activity. This is because water deficit reduces photosynthesis directly hence dehydrated protoplasm has a lowered photosynthetic capacity (Vurayai *et al.*, 2011). The decrease in Fv/Fm indicates to some extent the occurrence of photoinhibition due to photoinactivation of PSII centers possibly attributed to DI protein damage. The reduction in chlorophyll fluorescence among the NERICAS was more pronounced at water deficit during the reproductive stage as compared to vegetative stage. This may be attributed to the fact that at the reproductive stage the metabolic activities are faster and the plants absorbs and use more water as compared to vegetative stage hence low relative leaf water content. Low relative leaf water content may have predisposed the leaves to photoinhibition and the inhibition of photosynthetic activity could in fact reflect an inactivation of PSII activity and the concomitant uncoupling of non-cyclic photophosphorylation as shown in *Nerium oleander* (Bjorkman and Powles, 1984). Under well watered conditions, tolerant as well as susceptible genotypes maintained high Fv/Fm values but the varietal reduction of chlorophyll fluorescence during water deficit differed. NERICA 2 and 4 had the highest Fv/Fm ratio at water deficit treatment during vegetative stage and reproductive stage (Fig.7a and b). Maintenance of a relatively higher values of Fv/Fm by NERICA 2 and 4 during water deficit demonstrated that photochemistry of PSII, light driven electron transport and enzymatic reactions requiring ATP from chloroplasts were not severely affected compared to NERICA 1, 3 and 5 by the leaf water deficits induced by soil water depletion. The standard Fv/Fm ratio is 0.83 but typically ranges from 0.75 to 0.85 for normal healthy plants. The Fv/Fm ratio of the NERICA varieties recovered after rewatering in plants which were subjected to water deficit during the vegetative and reproductive stages. Similar results of plants lowering their Fv/Fm under water deficit and then recovering after rewatering were also obtained in beans (Miyashita *et al.*, 2005). The increase in Fv/Fm usually results in increase in dry matter production because of return to normal photosynthetic rates. Similar results of reduction of

Fv/Fm to mild drought stress make it a promising trait for screening for drought tolerance (He *et al.*, 1995). The electron transport rate was remarkably reduced by water deficit imposition (Table 5). The reduction was more pronounced at reproductive stage. Decrease in ETR among the NERICA varieties may be associated with increases in excitation energy quenching in the PSII antennae which are generally considered indicative of down regulation of electron transport. Estimates of ETR describe the ability of photosystems to use incident light thereby giving an indication of the overall photosynthetic capacity of the plant. In addition, the flow of electrons through PSII is indicative under many conditions of the overall rate of photosynthesis. The varietal difference was significant with NERICA 2 and 4 having a higher ETR rates at water deficit during vegetative and at reproductive stage. A possible explanation may be that compared to NERICA 1, 3 and 5, NERICA 2 and 4 were more tolerant to water deficit as low ETR under water deficit suggests low tolerance to water deficit.

CONCLUSION

In the present study, water deficit affected the growth, chlorophyll fluorescence and biochemical parameters that were measured on the 5 NERICA varieties. The effect was more pronounced at water deficit during reproductive stage as compared to vegetative stage. However, plant height and biomass accumulation were more sensitive to water deficit occurring at vegetative stage as compared to water deficit occurring at reproductive stage The study shows appreciable differences among vegetative and reproductive stages of growth in respect to their response to water deficit. Studies have demonstrated that many crops can tolerate water deficiency with no damage to plant growth or to any other physiological processes (Turner, 1990). This however is not the case with NERICA rice especially in its reproductive phase. The overall results indicate that there is genetic variability present in the NERICA varieties studied. NERICA 2 and 4 were tolerant to water deficit occurring at vegetative stage or reproductive stage as compared to NERICA 1, 3 and 5 because their growth, chlorophyll fluorescence, chlorophyll and protein content were less affected.

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