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CHLOROPHYLL FLUORESCENCE, PROTEIN AND CHLOROPHYLL CONTENT OF THREE NERICA RAINFED RICE VARIETIES UNDER VARYING IRRIGATION REGIMES

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ABSTRACT

Water deficit affects growth and development of rainfed rice leading to a considerable yield reduction or crop failure. Although the rice crop is susceptible to water deficit, there is a marked genotypic variation in protein and chlorophyll content in response to water deficit. The objective of this research was to investigate the effects of water deficit on chlorophyll fluorescence, protein and chlorophyll content of three recently developed rainfed rice varieties that is, New Rice for Africa (NERICA coded as N_2, N_4 and N_{11}) with a view of determining their tolerance levels to water d deficit. This study was carried out in the University Botanic Garden, Maseno during 2005- 2006. Plants were subjected to water deficit treatments in the green house and in the field in a factorial set up. The seeds of the three NERICA cultivars of rice were planted in 20 Litre PVC pots in the greenhouse and in the experimental plots in the field, arranged in a completely randomized block design (CRBD) with four treatments and four replications. The treatments included watering a litre of water once a day (control), Watering every 2, 4 and 6 days. The parameters measured included chlorophyll fluorescence, protein and chlorophyll content. The Water deficit had no significant effect on chlorophyll fluorescence parameters. The total chlorophyll and protein content declined with increasing water deficit. Results indicate that under moisture deficit conditions, there is no significant damage to the photosynthetic apparatus of the three rice varieties. NERICA 2 exhibited superior qualities indicating that it may perform well under water deficit conditions.

Keywords: rainfed rice, water deficit, growth, yield, chlorophyll fluorescence, protein, chlorophyll, NERICA rice.

INTRODUCTION

Water deficit is one of the most important environmental factors that limit crop production of rainfed agriculture in many parts of the world. Effects of water deficit on plants have been extensively studied and include osmotic, biochemical and physiological effects. Water deficit affects nearly all the plant growth processes; however, the stress response depends upon the intensity, rate, and duration of exposure and the stage of crop growth (Brar *et al*., 1990). Drought stress is a serious limiting factor to rice production and yield stability in rain-fed rice areas. In rice the effect of drought varies with the variety, degree and duration of stress and its coincidence with different growth stages (Kato *et al*, 2004). Rice is more susceptible to drought than other cereals because it is unable to regulate its transpirational water loss as effectively as other cereals (Austin, 1989). As a result droughted rice rapidly becomes damaged by the effects of low tissue water potential (Kato, 2004). Rice leaves in general have a very high transpiration rate, thus under high radiation levels rice plant may suffer due to midday wilting (Jongdee *et al.*, 1998). In Kenya is mainly grown under irrigation in the National Irrigation Board schemes of Mwea, Ahero, West Kano and Bunyala. In *vivo* studies of fluorescence provides basic information on the functioning of the photosynthetic apparatus especially photosystem II (PS II). An inverse relationship usually occurs between the photosynthetic activity and *in vivo* chlorophyll fluorescence (Pereira *et al.*, 2000). The Fv/Fm which reflects potential quantum yield of PS II indicates the physiological state of the photosynthetic apparatus in intact plant leaves (Pospisil *et al.*, 1998, Pereira *et al*., 2004; Maxwell and Johnson, 2000; Krause and Weis, 1991). Environmental stresses affect PS II efficiency and lower the Fv/Fm values (Pospisil *et al*., 1998). Studies by Randall *et al* (1977) on the consequence of drought stress on the organization of chlorophyll into photosynthetic units and on the chlorophyll-protein composition of mesophyll and bundle sheath chloroplast of *Zea mays* found that the majority of chlorophyll lost in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. All of the chlorophyll loss can be accounted for by reduction on the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al.*, 1977). Studies by Kura- Hotta *et al.* (1987) on rice seedlings showed that the chlorophyll content of leaves decreases during senescence suggesting that the loss of chlorophyll is a main cause of inactivation of photosynthesis. Potato leaves showed a significant decline in chlorophyll content with increasing water deficit (Nadler and Bruvia, 1998). According to Levitt (1980), chlorophyll content in plants often decreases with increased mesophyll resistance commonly observed in dry areas. Just about every aspect of cellular metabolism and fine structures has been reported to be affected by water deficit. Particular characteristic changes include; increases in rates of degradative as compared with synthetic reactions (Mckersie and Ya'acov, 1994). Water deficit impedes protein synthesis perhaps at the ribosomal level; some proteins are apparently formed and inactivated quickly whereas others appear to be relatively stable (Lutts *et al.*, 1996). In many plant tissues a reduced water

potential causes a reduction of total protein synthesis and a rapid dissociation of polyribosomes (Jones, 1989 and Bardzik *et al.*, 1971). Studies on sunflower by Rao *et al.* (1987) showed that water deficit reduces seed protein content. Electron microscopy of wilted willow leaves indicates that under dehydration partial protein crystallization takes place in the chloroplast stroma (Vapaavuori and Nurmi, 1982). Crystallization should decrease enzyme activity, since catalysis takes place only at crystal surface (Kaiser, 1987). Protein content, particularly of soluble protein usually falls to about 40-60% of the initial content as water deficit becomes intense in drought sensitive plants (Lutts *et al.*, 1996). Protein synthesis may be particularly sensitive to water deficit for example most polysomes revert to monosomes in four hours at -0.5 MPa in *Zea mays* roots (Kaiser, 1987). Reduced protein synthesis appears to stem partly from diminished RNA synthesis and partly from a four fold increase in RNase activity at 43% relative water content because water stress enhances the synthesis of one of the alpha-amylase isozymes (Jones, 1996). This study was undertaken to investigate the effects of water deficit on chloropyll fluorescence, protein and chlorophyll content of three NERICA rice varieties and to determine the varietal difference in response to water deficit.

MATERIALS AND METHODS

This study was carried out at the University Botanic Garden, Maseno in the green house. Maseno University is situated in Western Kenya. The area receives a mean annual precipitation of 1750 mm with a bimodal distribution. The mean temperature of Maseno is 28.7^{O}C and it is approximately 1500 m above sea level. Maseno lies at latitude 0^{o}1'N - 0'12'S and longitude 34^{o}25'E-47'E. The soils at Maseno are classified as acrisol being well drained, deep reddish brown clay with pH ranging between 4.6 and 5.4 (Mwai, 2001). The potted plants were grown in a naturally illuminated green house where the light, CO_2 concentration and temperature conditions were uncontrolled. Day temperature ranged from 20 - 40^{O}C, relative humidity, 45 - 90%. Maximum photosynthetic photon flux density (PPFD) or Photosynthetic active radiation (PAR) ranged between 250 - 600 µmol $m^{-2}s^{-1}$, measured at the upper leaf surface. The natural light intensity was not supplemented. Air circulation in the green house was maintained by partially opening the windows. The study was conducted between September 2005 and July 2006. Seeds of 3 NERICA rice *(Oryza sativa* L.), varieties namely NERICA 2, 4 and 11 coded as N_2, N_{11}, and N_4 were obtained from the NERICA adaptability trials in the University Botanic garden, Maseno. The seeds had been developed for rain-fed culture by African Rice Centre in West Africa. The soil was dug from the garden then solarized for one week after which the soil was filled into 20 litre PVC pots with perforated bottoms up to ¾ full. The seeds were soaked for a day prior to planting to facilitate germination. The three NERICA varieties were planted with 4 treatments and 4 replications. Diamonium phosphate (DAP) Fertilizer was applied in the pots during planting at recommended rate of 52 kg/ha. Top dressing was done using Calcium ammonium nitrate (CAN) fertilizer in split application of 26 kg/ha at 21 days and 26 kg/ha at panicle initiation. The experimental design was a completely randomized block design (CRBD). The pots were watered to field capacity before planting. After germination the pots were irrigated daily with one litre of water until they were three weeks old. The watering regimes were; watering once in a day (control), watering after every 2 days, watering after 4 days and watering after 6 days. Four seeds per hill were sown and there were 4 hills per pot with a spacing of 15 x 25 cm. The pots were kept weed free by hand picking the weeds.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were carried out on intact leaves with a portable fluorescence monitoring system (Hansatech model FMS 2; Hansatech Instruments, Germany). Measurements were done on the upper unfolded leaf of the main tiller between 1000 and 1200 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. The initial fluorescence (Fo) and the maximum fluorescence level (Fm) were measured. The variable fluorescence (Fv) was calculated as (Fv = Fm - Fo) and the maximal quantum yield of PS II photochemistry (Fv/Fm) was determined.

Chlorophyll content determination

Chlorophyll content was determined on the flag leaf using methods of Arnon (1949) and Coombs *et al* (1987). One gram of the fresh leaf tissue was cut into small pieces and placed into a specimen bottle containing10 ml of absolute ethanol and 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 of the chlorophyll solution measured using a spectrophotometer (Ultrospec 11) at 645 and 663 nm. The chl.a and chl. b content in milligrams (mg) were estimated using the formula of Arnon (1949).

Protein content determination: One gram of the fresh flag leaf tissue was weighed and cut into small pieces into specimen bottles then mixed with 10 ml of 2% anhydrous Sodium Carbonate in 0.1 M Na0H and stored for one month. 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 then allowed to stand for 15 minutes at room temperature after which 0.5 ml of Folin-Ciocalteau reagent was added to the solution and 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 then estimated by the Lowry method as described by Coombs *et al*. (1985) using bovine serum albumin as a standard.

Statistical analysis

Analysis of variance (ANOVA) was done using a statistical computer package (SAS) to determine the effects of watering regimes. The treatment and variety means were separated using the least significant differences (LSD) test at 5%level.

RESULTS

Chlorophyll fluorescence

The first two watering regimes did not show any dramatic change in the quantum yield as indicated by Fv/Fm ratio which was above 0.8 for the three varieties of rice in experiment 1. Some differences though not significant occurred at the 3rd and 4th watering regimes. At the 4th and 6th irrigation regimes some noticeable differences occurred among the three varieties where N_2 had the highest Fv/Fm values. N_{11} had the lowest quantum yield at 6th watering regime in Experiment 1 and 4th and 6th watering regimes in experiment ll.N_2 had the highest Fv/Fm values in plants watered after 4 and 6 days during the booting stage (at DAS 74) in both experiment 1 (Figure-1a) and in experiment II (Figure-1b) while N_{11} was the most affected by the water deficit during the booting stage and had the least Fv/Fm values in plants watered after every 4 and 6 days (Figures1a and 1b).

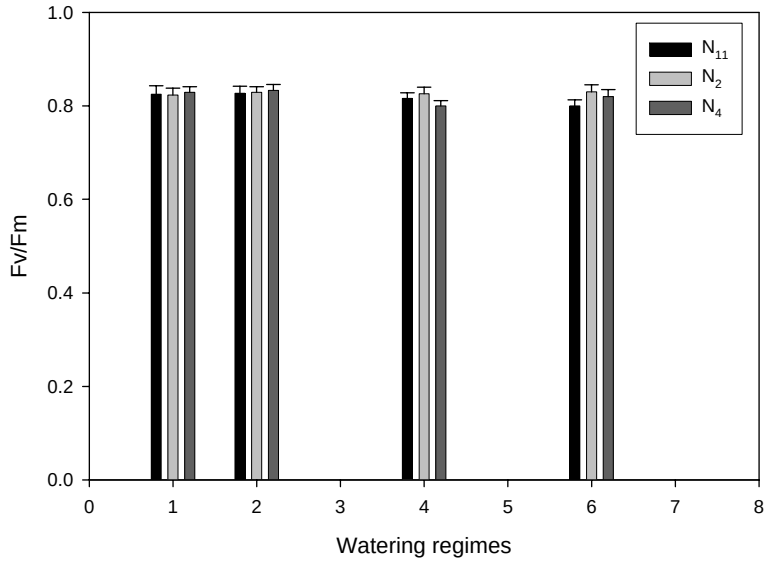

Figure-1a. Effects of different treatments on Fv/Fm ratio at DAS 74 in experiment 1. Each point represents the mean of four treatments ± STD DEV.

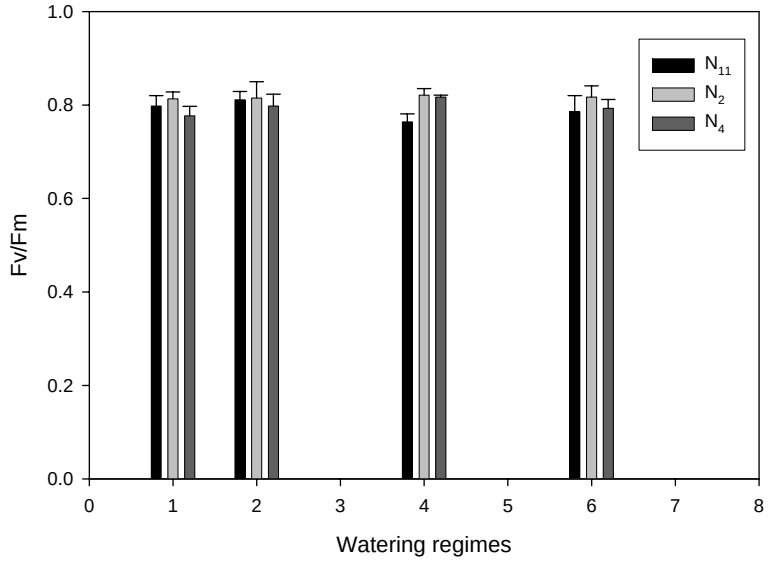

Figure-1b. Effects of different treatments on Fv/Fm ratio at DAS 74 in experiment II. each point represents the mean of four replications ± STD DEV.

Chlorophyll concentration

N_{11} had the lowest chlorophyll (chl.a) content in plants watered after every 2, 4 and 6 days (Figure-2a). It was also the most affected by water deficit for chl.b and total chlorophyll (Figure-2c). N_2 had the highest chlorophyll b (chl.b) content in plants watered after every 2 and 6 days (Figure-2b) and the highest t chl. in plants watered after every six days (Figure-2c). N_{11} had the lowest chl.b content in plants watered after 1, 4 and 6 days. There was no significant difference ($P>0.05$) in chl.a and t chl. content between N_2 and N_4 though they were significantly different from N_{11}.

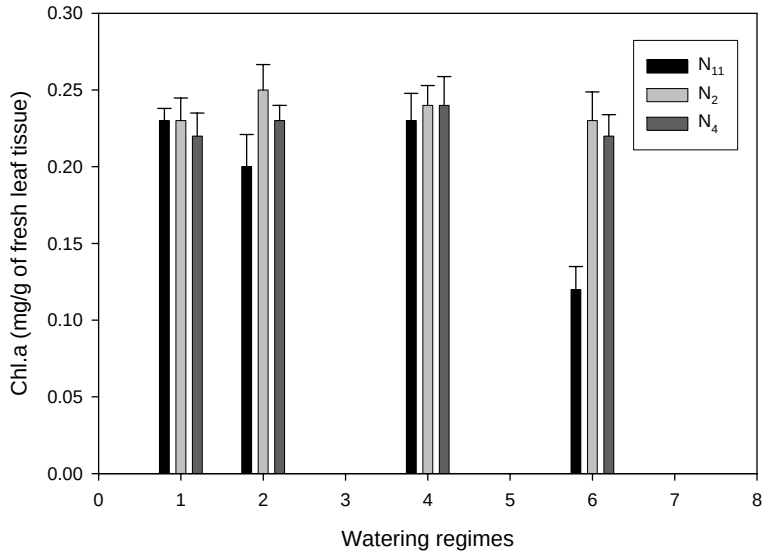

Figure-2a. Chlorophyll a content of three NERICA rice varieties subjected to four treatments. Each point represents the mean of four replications ± STD DEV.

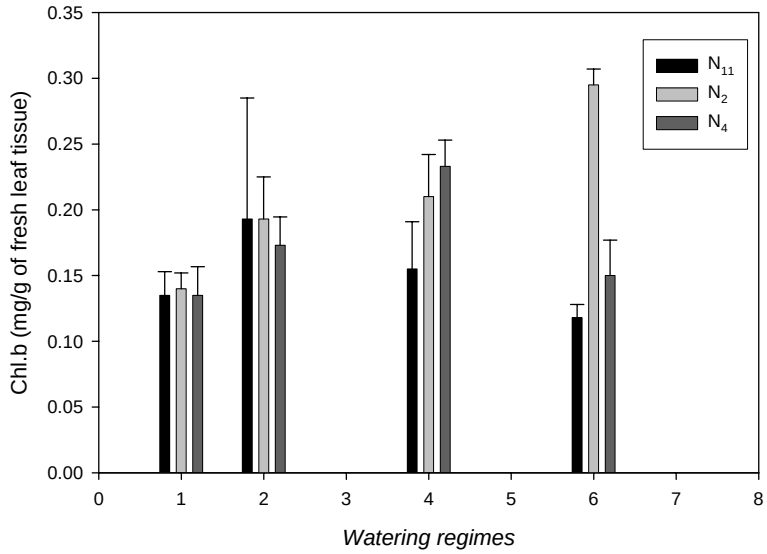

Figure-2b. Chlorophyll b content of three NERICA rice varieties subjected to different treatments. Each point represents the mean of four replications ± STD DEV.

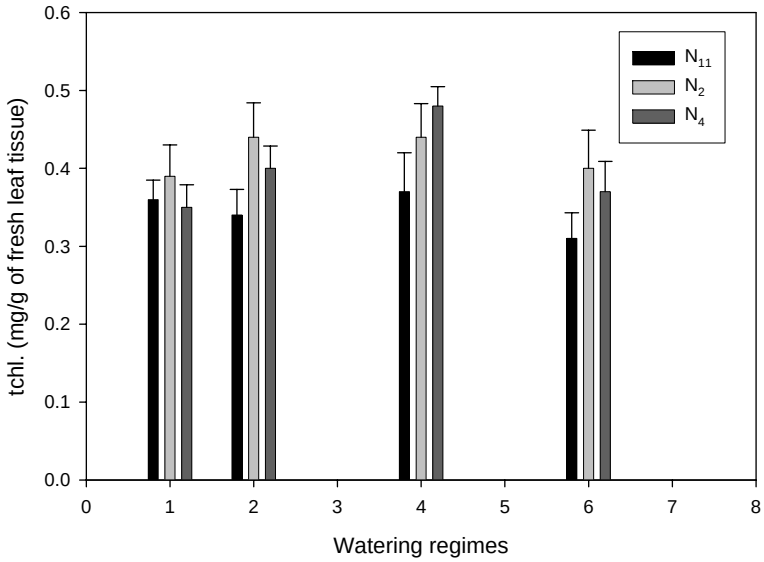

Figure- 2c. Total chlorophyll content of three NERICA rice varieties subjected to different treatments. Each point represents the mean of four replications ± STD DEV.

Protein content

There was a general reduction in protein content with increased water deficit. N11 was the most affected by water deficit and recorded the highest reduction in protein content with increased water deficit. The protein content in N2 declined in plants watered after every two days but was constant between the plants watered after every 4 and 6 days as shown in Figure-3.

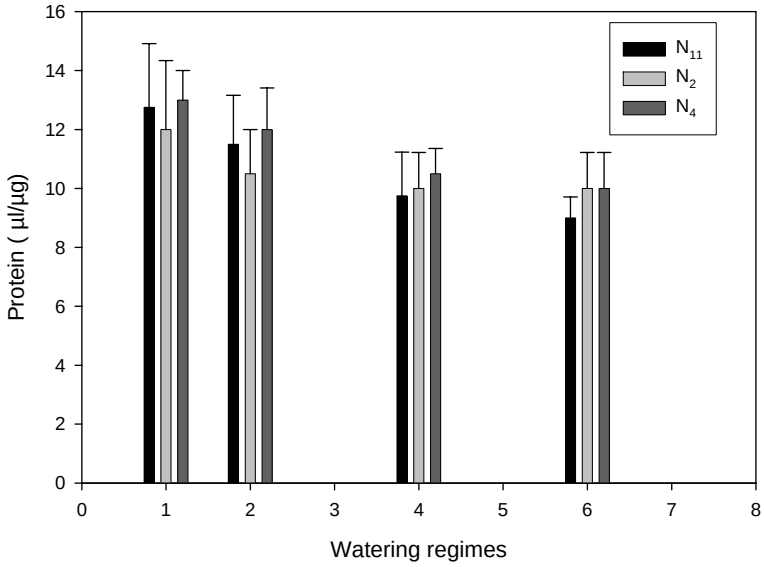

Figure-3. Effects of different treatments on the protein content of three NERICA rice varieties. Each point represents the mean of four replications ±STD DEV.

DISCUSSIONS

The standard Fv/Fm ratio is 0.83 (Demmig and Bjorkman1987). The current results fall within the limits. Similar results have been reported by Cornic and Massaci (1996), Ohashi *et al.* (2004), Baruah *et al.* (2006) and Lu and Zhang (1999). The results indicate that leaf photochemistry was rather resistant to water deficit and electron transport chain was maintained under water stress conditions (Ohashi *et al.*, 2004). This implies that the water deficit levels do not change the functioning of PS II in the absence of photorespiration (Cornic and Massaci, 1996). Drought does not induce a significant increase in alternative pathways of electron transport particularly that involved in the direct reduction of oxygen (Cornic and Massaci, 1996). The regulation of PS II activity at normal oxygen molar fraction is the same when photosynthesis is changed by leaf water status and that stomatal closure during dehydration cause the observed decline in leaf

photosynthesis (Lu and Zhang 1999). The photosynthetic apparatus of N_2 seems to be the least affected by water deficit compared to N_4 and N_{11} as indicated by the high Fv/Fm values even under soil moisture deficit.

The observed reduction of chlorophyll in water stressed plants may be due to a reduction in the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al.,* 1977). Similar results have been reported by Kura-Hotta *et al.,* (1987) and Nadler and Bruvia (1998). Photosynthetic capacity of a plant is determined by several factors including photosynthetic pigment composition (chlorophyll content). The efficiency of light captured to drive photosynthesis is directly correlated to the chlorophyll concentration in the leaf (Netondo, *et al*., 2004). In this study N_2 recorded an increase in chlorophyll b content with increase in soil moisture deficit and had a higher content of chlorophyll a, chlorophyll b and total chlorophyll than N_4 and N_{11} in the highest soil moisture deficit watering regime. Thus N_2 seem to be more tolerant to dehydration than N_4 and N_{11}. The maintenance of a higher total chlorophyll under water deficit suggests that there was less destruction of chlorophyll pigments in N_2 leaves during water deficit and chlorophyll synthesis was induced by water deficit especially chlorophyll b (Luvaha, 2005).

The protein content among the NERICA varieties reduced with the decrease in soil moisture content. The results are in agreement with those of Bewley and Dasgupta (1984) and Rao *et al*. (1987). Water deficit in plant tissue may cause reduction in polyribosome levels or lead to increase in the protein breakdown (Onyango, 1996). Leaf protein function not only as a catalyst but also a major storage sinks for nitrogen. Some proteins such as RuBP carboxylase act as both catalyst and as storage protein. The turn over characteristic of individual protein depends a great deal on their intracellular location and their accessibility to leaf protease (Onyango, 1996). Whereas N_2 recorded constant protein content between plants watered after every 4 and 6 days. N_{11} was the most affected by water deficit and the protein content significantly reduced with increase in water deficit.

CONCLUSIONS

In conclusion irrigation regimes imposed to elicit water deficit had minimal impact on the quantum yield. However, some decrease in protein and chlorophyll contents occurred.The NERICA (N_2), seem to show some superior qualities of being tolerant to water deficit conditions compared to N_4 and N_{11}. Further studies are recommended to establish why its chlorophyll and protein content are less affected.

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